Manual

of

Alcoholic Fermentation

And the Allied Industries

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PREFACE

THE progress of the industries to which science is a handmaid has become so rapid that, figuratively speaking, the discoveries of to-day speedily eventuate in the well-proved method or system of to-morrow, and it is thereby borne in upon us that whilst science and scientific research give a true stimulus to the various technologies, these, in their turn—owing to the earnestness of purpose and desire for knowledge of the individuals who guide and control them—stimulate research. This can be very truly affirmed of the industries in which fermentation plays the most important rôle, for from the very earliest times they have afforded scope for speculative thought and scientific experiment, and have not merely emerged from the region of mere customary or traditional procedure, but have been placed on a sound basis of ascertained fact, and by virtue of this have become truly scientific industries.

The rapid rate at which fresh knowledge is continually being added to the store, and the increasing complexity of many of the issues involved, require a mind well trained in scientific modes of thought, otherwise the varied phenomena which are presented by the chemistry of fermentation on the one hand, and the life-history of the agents which cause fermentation on the other, cannot be effectively grappled with.

Any one who has been brought into contact with the fermentation industries can hardly have failed to perceive how absorbingly interesting they are, for even the most superficial observation renders it apparent that not only are the ascertained facts of marked interest, but that an almost

inexhaustible field of work is open to the experimenter, whether it be of the nature of purely scientific research or the attempted solution of problems suggested by practical considerations.

An attempt has been made by the author in the scope of this manual, not to provide a treatise on chemistry, wine-making, brewing, and distilling, but to deal with alcoholic fermentation from the point of view of modern science and theory, and also to show how modern scientific discovery has reacted on the alcoholic fermentation industries, and to give some indication of the advance which has in consequence been made. It is a safe prediction that this advance in knowledge will go on with increased rapidity, whilst the necessity for a very thorough equipment of scientific knowledge on the part of the technologist will increase with it. It is wellnigh certain that in a very moderate time—if it is not so already—the science of fermentation will include issues very difficult to grasp and understand without a very intimate acquaintance with the knowledge that has been already acquired, and a clear perception of its bearings. The author hopes and believes that what has been introduced into this work will not only be of value to the student but also to the practical technologist, and that in the case of both it will serve as a stimulus to the acquisition of the large amount of valuable information lying beyond in the various excellent works which deal more minutely with the separate technologies in which alcoholic fermentation plays so important a part.

The author wishes to express his cordial thanks to his brother-in-law and partner, Mr. Frank Edward Lott, for his kind help in correcting the MS. and for valuable suggestions in connexion with the body of the work.

BURTON-ON-TRENT,
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Malt and Malting	H. STOPES.
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Handbuch der Spiritusfabrikation	MÄRCKER.
Presshefefabrikation	OTTO DURST.
The Microscope in the Brewery and Malthouse	MATTHEWS & LOTT.
Origin, Nature, and Use of Wine	THUDICHUM & DUPRÉ.
Die Weinbereitung	PIAZ.
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Transactions of the Northern Institute of Brewing.	
Transactions of the Midland „ „	
„ „ Laboratory Club, Institute of Brewing, and Federated Institutes of Brewing.	
Betriebskontrolle in den Gährungsgewerben	LINDNER.
Untersuchungen über die Alkoholgährnngspilze	Dr. MAX REESS.
Les Levures	KAYSER.
The Soluble Ferments and Fermentation	T. REYNOLDS GREEN.
Études sur la Bière	PASTEUR.
Études sur le Vin	„
Theorie der Gährung	C. v. NÄGELI.
A Handy Book for Brewers	H. E. WRIGHT.
Chemie und Physiologie des Malzes und des Bieres	Dr. EUGEN PRIOR.
Theory and Practice of Modern Brewing	FAULKNER.
The New Chemistry	COOKE.
Organic Chemistry	ARMSTRONG.
„ „	WHITELEY.
„ „ part iii, section i, Miller's Elements	ARMSTRONG & GROVES.
Catalogue of Brewing Appliances	LLEWELLYN & JAMES.
The Brewing Journals.	

ALCOHOLIC FERMENTATION

CHAPTER I

ALCOHOLIC FERMENTATION. GENERAL CONSIDERATIONS LEADING TO SPECIAL ONES

THE word fermentation is a decidedly interesting one on account of its associations, for it is but a step in one's thoughts from the process to the product, and it is in the products of fermentation, naturally, that general interest centres. Those who have enjoyed the vintages of the Rheingau on board a Rhine steamer, or have partaken of the wines of France during some pleasant holiday spent on French soil, can associate themselves with this idea; and even if it come to humbler, but equally popular beverages, such as 'Lager' or 'Bock' beer and our English ales, there is much still to be said as regards pleasurable associations in connexion with their reasonable use and enjoyment.

The discovery of fermentation was probably a fortuitous circumstance, and very likely resulted from the hardihood displayed by some ancient experimenter, in drinking the result of the spontaneous fermentation of some saccharine liquid which had been left exposed to the air. A certain degree of exhilaration experienced would lead to a repetition of the experiment and to a regular sequence of such brewings. Man in a state of primitive savagery seems to have a craving for something that either intoxicates or stupefies, or which perhaps does both, for most native tribes seem to prepare something of this nature, and the fermented substances—they can hardly be called beverages—would appear in many cases to be of the very crudest description.

The prime desideratum in a fermented liquid is unquestionably the alcohol, and no process of change in a liquid by which alcohol is not produced can be truly described as fermentation; though the word

signifies more especially, perhaps, whatever in the process is visible to the eye, that is to say the movements of a fermenting liquid, as also changes in appearance and odour, and the formation of a 'head,' followed by settling and partial or complete clarification of the bulk. For a long time past yeast has been used to excite fermentation in saccharine solutions, the yeast accruing from one operation being employed as the means of starting another. Prior to the intentional addition of yeast, fermentation took place either naturally or fortuitously, in fermentable liquids, as, for instance, in the expressed juice of the grape or of other fruits. All saccharine liquids may be regarded as being susceptible of fermentative changes, and especially the liquids provided in nature of the kind just mentioned, the character of the fermented product being dependent—speaking generally—on the substances yielding the fermentable fluid. Some of the earliest records of fermented beverages relate to those produced from preparations or infusions of cereals, or liquids of the nature of beer. The ancient Egyptians were acquainted with a beverage of this character made from barley, and allusions to it are made in ancient papyri. The Jews were evidently acquainted with the fermentation of dough by the use of leaven, and there is some reason for believing that the leaven thus employed was the outcome of some kind of fermented beverage. Beer was known to the Romans under the name of *cerevisia*, and was doubtless prepared from barley or some other cereal. At the present time a primitive kind of beer is made from malted maize by the Peruvians: it is termed *chica*, and results from the spontaneous fermentation of the saccharine extract of the maize, and carries a good deal of the unpleasant flavour so noticeable in certain kinds of Belgian beer produced by this kind of fermentation.

The derivation of the word 'fermentation' from the Latin *fermentum* is clear as describing the action of yeast in dough and in liquids susceptible of change in appearance and flavour; in the one case there is the *puffing up* or *swelling* of the fermenting dough, in the other there is the production of vinous flavour usually accompanied by a perceptible lessening in density or *thinning* of the liquid as it ferments. The Latin word *fermentum* means these things or, at all events, some of them, and if, as is supposed, the verb *fervēre* (to boil) is the root word, even then the meaning is realized in the case of certain fermentative operations on the industrial scale—such as distillers' fermentations—in which the evolution of gas is so rapid as to throw the whole volume of liquid into movement and make it appear to boil.

One of the most familiar instances of fermentation and its results is furnished by ordinary stone-bottle gingerbeer, which, if only containing the small amount of alcohol permitted by the Excise, may be classed among the beverages which cheer but do not inebriate. The presence of alcohol and other products of fermentation is perceptible to the palate, whilst the mild explosion that accompanies the parting of cork and bottle tells of the gas which has been produced, as also does the further escape of bubbles from the poured-out liquid. The preparation of home-made yeast or ferment for bread-making, and the production of home-made wines, are based upon natural fermentative processes fostered or elaborated by artificial methods.

We are now in a position to bring the matter to the point of a general definition as follows:—Fermentation proper is an action which takes place in a liquid or pasty substance giving rise to definite changes in taste and smell of a more or less agreeable character, and possibly changes in appearance, alcohol and carbonic acid gas being produced at the same time.

If a liquid in an active state of fermentation be filtered, the yeast in suspension may be removed; and if this be completely effected fermentation ceases. The yeast thus removed is capable, as is well known, of starting a fresh fermentation: or it may be carefully dried off at a temperature below 100° F. and retain its power for a considerable period, but the temperature of boiling water, or a short exposure to a temperature exceeding 130° F., as well as exposure to a very great degree of cold, destroys its fermentative action. Saccharine solutions which have been boiled and subsequently exposed to the air not unfrequently enter into alcoholic fermentation; but if, whilst hot, the containing vessel—a glass flask, for instance—be plugged with cotton wool, the liquid remains practically unaltered. We are thus in possession of evidence as to the following points:—

1. That there is something in yeast which causes fermentation.
2. That this property of yeast is destroyed by a high temperature.
3. That the property is associated with particles which may be separated from the fluid containing them by an efficient filter.
4. That these particles may be contained in the air, and may be separated from it by causing it to pass through cotton wool.

Microscopical examination of a drop of yeast shows what the particles in question are: they are definite organized cells.

A certain amount of confusion has arisen from the application of the words 'ferment' and 'fermentation' to processes which are not in

accord with the general definition of fermentation which we have given, and which in our view cannot properly be regarded as fermentative processes, and would not be so regarded by the majority of people. The misapplication takes two forms more especially, the first one being this:—Certain chemical changes are known which are brought about by substances which have been produced by, or in association with vital processes, but which have no vitality themselves. An illustration of this is furnished by the action of malt diastase on gelatinized starch, or of pepsin in a feebly acid solution on egg-albumen; in both cases a solvent action is shown and the substances causing the dissolution of the starch paste on the one hand, and the egg-albumen on the other, have been and still are spoken of as unorganized ferments; but if the matter be referred to our definition, inasmuch as they do not and cannot produce alcohol and carbonic acid gas in a fermentable solution, they cannot properly be described in this way, however remarkable or interesting the transformations they are able to effect may be.

The second form of misapplication is the following:—The minute organisms—microbes or bacteria—which accompany all putrefactive decompositions and a variety of ailments or diseases of men and animals, initiate processes which have been described as fermentative. As regards one or two possible features of their action, and in a general sense, the term seems not wholly inaccurate, as unquestionably there may be a certain resemblance to fermentation proper; at the same time it must be remembered that whatever else may be produced by these organisms, alcohol is not one of the products, and carbonic acid gas is seldom produced by them in the plainly visible manner in which it is evolved from an actively fermenting liquid. It seems then that if the term 'fermentation' is to be employed in connexion with the growth of putrefactive organisms, it should be strictly qualified by prefixing the name of the chief product of the action, and even then it is open to objection. It seemed desirable to clear the ground thus early, and it will undoubtedly conduce to clearness of meaning if it be borne in mind that by 'fermentation' we imply the action of a definite agent—an organism—capable of producing alcohol and carbonic acid at least, when brought into contact with a fermentable solution, whatever other substances may be simultaneously formed.

Let us now proceed to trace some of the results of scientific investigation into the phenomena attending fermentation, and see, as in many other cases, how the edifice of knowledge in this particular branch of

science has been built up stone by stone. One side of the matter has been elucidated by the use of the microscope as an instrument of research, whilst the other has been illuminated more especially by chemical science. As early as 1680 the great microscopist Leuwenhoek had discovered that beer-yeast consisted of very small spherical or ovoid globules: further than this, however, he did not get, and it was not till another century had elapsed that Fabroni (1787) added some important information. Fabroni regarded fermentation as a purely chemical process, and regarded yeast as a substance allied to the gluten of plants. He showed that fermentation could not be induced in uninjured fruits, and drew from this the correct conclusion, that in order that fermentation may be induced, the ferment and the fermented body must be in actual contact. He imagined that the carbonic acid gas evolved during fermentation came from the ferment and not from the fermenting substance, also that the alcohol was not pre-existent in the fermented fluid, but only arose during the distillation. At the beginning of the present century Thénard made an important contribution to the science of fermentation. He arrived at the conclusion that all natural juices in which fermentation has taken place give a deposit which has the appearance of beer-yeast, and is able to ferment pure sweetened water. He also found that yeast resembled animal matter in its chemical composition, especially in being so rich in nitrogen. Gay-Lussac about 1810 propounded a theory that oxygen was the sole cause of all fermentation phenomena, whether of alcoholic fermentation, putrefaction, or decay. He considered that the access of oxygen set the particles of the ferment in a state of movement, and that this movement was communicated to the more stable fermentable substance. In 1835 Cagniard de Latour, a French chemist, took up this line of research and found that yeast was a mass of organic globules capable of reproducing themselves by means of buds, thus appearing to belong to the vegetable kingdom. He concluded that in the course of vegetation the yeast disengaged carbonic acid gas from the saccharine liquid and converted the latter into a spirituous liquor. An independent but almost simultaneous discovery of the same fact was made two years later (1837) by Schwann at Jena and Kützing at Berlin, and it was firmly established that yeast was a mass of organized and living cells composed like the cells of animal or vegetable tissue of an envelope and granular contents. It was, however, not agreed what place should be assigned to the newly discovered organism, some considering it

to be a fungus without a mycelium, whilst others regarded it as an alga. The name of Turpin should not be excluded from the group of investigators of this period, as in 1838 he demonstrated the presence of living organisms in a fermenting liquid and connected the fermentation with the organisms. About this period, too, Meyen concluded that yeast was a fungus and created a new genus for it under the name of *Saccharomyces*, and this name has received general adoption. From about the date of 1840, very little was added for more than a quarter of a century except in the way of theory from the great chemist Liebig, and other scientists of a similar school of thought—such as Fremy and Trécul. Traube in 1858 put forward a theory rather resembling Gay-Lussac's view. It was this:—in the interior of each fermentation organism a substance is formed, allied in its nature to proteids and having a powerful affinity for oxygen; this substance assists the progress of fermentation by taking oxygen from one group of substances and transferring it to another. In 1870 a great advance was made when Dr. Max Reess published his *Botanical Investigations on the Alcoholic Ferments*[1], in which after a preliminary reference to the confusion that had arisen from calling organisms that were very diverse in their actions and products yeasts, he proceeds to narrow the term to the description of the alcoholic ferments proper, and then deals with Saccharomyces Cerevisiae of beer fermentation as conducted on the 'high' and 'low' temperature systems respectively, and further gives much information concerning some five well-established species of yeast and one or two less well-known ones, and their modes of growth and reproduction. Meanwhile Pasteur had been actively engaged in research on questions connected with fermentation, having apparently been drawn in this direction—as he undoubtedly was in other of his great researches—by the desire to benefit the arts, manufactures and industries of his country. He first turned his attention to wine, and whilst studying certain maladies or diseases—if one may use the term—that it is liable to, and which in certain years were the cause of heavy loss to growers and merchants, he discovered many facts of great importance in connexion with fermentation generally. Pasteur's discoveries and observations as regards wine were summed up in his work, *Studies on Wine*[2], published in 1872. By this time it was becoming a generally accepted fact that fermentation was the

[1] *Botanische Untersuchungen über die Alkoholgährungspilze*, Dr. Max Reess.
[2] *Études sur le Vin*, L. Pasteur.

result of the vital processes of a definite organism of a vegetable nature, and when Pasteur's monumental work, *Studies on Beer*[1], appeared in 1876, this position was unassailably established. Pasteur's conclusions still found, however, a strong opponent in the great chemist Liebig, who for years had contested the vital theory of fermentation. Liebig referred the whole of the phenomena of fermentation to a continuous chemical decomposition set in train by the putrefaction or decomposition of animal and vegetable matters; such putrefaction being communicable to adjacent substances. He thus ignored the action of a specific organism, and though compelled at length in face of Pasteur's researches and the conclusions of other scientists to modify his views, he never formally withdrew them. Dumas contributed some very important information about the time of the Pasteur-Liebig controversy. By some experiments, devised and executed with great scientific ingenuity, he succeeded in proving (1) that saccharine liquids are not influenced by yeast excepting by direct contact; (2) that the smallest interval in space between the ferment and the fermentable liquid precludes fermentation, as does also the separation of the yeast from the fermentable liquid by the thinnest membrane. These results lead to the direct inference that fermentation is not due to a transmitted movement, as Liebig insisted. It is not uninteresting to summarize the various views which have been expressed during the whole period passed under review:—

1. Fermentation as an effect resulting from the growth or vegetation of an organism. (The accepted theory as established by scientific knowledge.)
2. A mechanical theory or theory of chemical decomposition. (Liebig's theory, and that of the Liebig school.)
3. A theory of so-called catalytic action or decomposition by contact—presumably of the ferment and fermentable substance. (An elegant mode of expressing ignorance of the true action.)

One theory or opinion remains worthy of mention, and it is that of the chemist Berthelot, who considered that fermentation is produced by the action of a substance manufactured by organic ferments. Viewed in the light of recent discoveries which will be spoken of in another chapter, we shall see what a very important hypothesis this was.

It can hardly be doubted that in organic living cells, whether they

[1] *Études sur la Bière*, L. Pasteur.

be simple ones such as yeast, or existing as integral parts of a compound organism, there are forces at work which give rise to chemical actions which cannot be imitated in the laboratory, but the idea of such vital forces acting chemically is a very different one to Liebig's idea of the spontaneous decomposition of some nitrogenous substance. The latter implies—what is opposed to ordinary scientific discernment—that a substance has some innate capacity of developing force to upset its own chemical equilibrium. The fact of the matter would appear to be that Liebig had no conception of the part that is played by microscopic living organisms in nature, which are really responsible for the very putrefaction he speaks of. The actual changes in composition seemed to be the only things that attracted Liebig's notice.

Sufficient has now been said to show that the weight of evidence is overwhelmingly in favour of the view that fermentation is the visible result of the growth of an active living agent, and that apart from the results of the vital processes of the yeast organism or other living cells the production of alcohol from a saccharine liquid is unknown. Let us now turn our attention to the question of the occurrence of the yeast organism in nature. It is plain, in the first place, that fermentation—even of a fortuitous or spontaneous kind—is of such common occurrence that it implies a certain plenteousness of the living cells that are able to bring it about; and so perhaps the first question that would naturally be asked is, Where do the ferment cells come from? Simple reasoning would lead one to infer that they are either to be found on, or adjacent to, the fruits furnishing readily fermentable saccharine liquids or juices; or that they reach these liquids from the atmosphere. Both these inferences are correct, but the ferment cells that are found on ripe fruits are present in such abundance that the other source as a means of initiating fermentation at any given time sinks almost into insignificance in comparison with it. Pasteur and Reess showed most conclusively that grapes—for example—carry on their surface the cells of various organisms amongst which ferment cells preponderate; these cells constitute the 'bloom' of the grape, and it is apparent that some kind of attraction or some selective process not fully understood, causes the ferment cells to thus gather on the fruit and hold themselves, as it were, in readiness for the opportunity that generally comes, of exchanging a dormant condition for one of great activity what time the living ferment cells become submerged in the fruit juice either accidentally, as by the bursting of

the skin of the ripe grape, or the intentional bruising and breaking up that takes place in the wine-press. In the last case the grape juice or 'must' flows forth carrying suspended in it countless ferment cells from the skin of the grape, so that active fermentation follows in the well-known manner. What holds good for the grape holds equally for a great many other fruits, and so it will be readily understood why spontaneous fermentation—that is fermentation as apart from the intentional addition of yeast—results so speedily. Somewhat recent investigation has shown that the soil in proximity to vines, fruit-trees, &c, becomes a harbour for ferment cells, owing to the fact that fallen ripe fruit carrying ferment cells on its surface becomes intermingled with earthy matter and the ferment cells thus buried remain torpid through the winter months, to be wafted into the air as dust in the spring, and to find their way eventually on to the ripening fruit. The point that remains unexplained is, why the living cells should preferably attach themselves to the fruit, unless indeed it is that they are pretty equally distributed on the vines or fruit-trees but are more plainly discerned on the fruit. It is to be believed, however, that they are really more abundant on the fruit. The occurrence of a moderate quantity of yeast cells amongst the heterogeneous floating matter of the air will—in view of what has been just said—not appear very remarkable; and it can be realized at the same time that a fermentation inaugurated in such a way is, in the majority of cases, necessarily of a mixed or impure character.

CHAPTER II

ALCOHOLIC FERMENTATION AS A PROPERTY OF LIVING CELLS

IN the preceding chapter a considerable amount of evidence was adduced as to fermentation being the result of the growth of a specific living organism. In reality, no fact has been more clearly demonstrated than that without the presence of a ferment cell ordinary fermentation cannot take place. The statement is, however, susceptible of modification in two ways. First, under certain conditions the living cells of plant tissues are able to act as ferments. Lechartier and Bellamy found that if ripe fruits (pears, apples, cherries, &c.) were placed in tubes or test-jars standing over mercury in a trough, that the whole of the oxygen of the enclosed air was absorbed by the fruit. This absorption is accompanied and followed by a considerable production of carbonic acid gas. If the air be renewed, absorption of oxygen and production of carbonic acid again go on, and this may continue for many months. On breaking up and distilling the fruits alcohol is obtained in notable quantity, although no yeast organism is discoverable in the internal portions of the fruit, and the action is clearly due to some other influence. Pasteur verified these results by experiments of his own, and the facts unmistakably prove that vegetable cells are able to produce alcohol and carbonic acid at the expense of sugar: that they can indeed act like ferment cells proper, only much less energetically, the action being probably due to some phase of the residual vitality in the cells of the fruit tissue.

The second modification of the accepted fact that fermentation is the immediate result of the life processes of a definite organism is the following:—It has been rather recently discovered by Buchner that if active yeast be submitted, under suitable conditions, to enormously high pressure, a liquid can be expressed from it which is able to cause visible fermentation in a saccharine solution, giving rise to alcohol and

carbonic acid gas. A further allusion will be made to this truly remarkable discovery.

Let us now proceed to take a very general view of the nature and composition of the yeast organism. If a small quantity of pressed or baker's yeast be heated in a silver or platinum dish it browns and partially melts, and very speedily a cheesy odour is distinctly perceptible: as the burning proceeds, the smell passes through various stages of unpleasantness till it resembles burning animal matter. Heated still further, a black mass of charcoal or carbonaceous matter is left which, if completely incinerated, is reduced to a white or greyish-white mass consisting entirely of mineral matter. The experiment as a whole demonstrates the fact that organic and inorganic matters enter into the composition of the yeast cell. Chemical analysis shows that yeast contains carbon, hydrogen, oxygen, and nitrogen, with relatively small quantities of sulphur, phosphorus, potassium, magnesium, and calcium. These elementary substances are variously combined to form the constituents of the yeast cell, viz. albuminoid or proteid matter, carbohydrate matter, fat, saline substances, and water. The envelope of the cell represents the carbohydrate portion more especially, consisting, as it undoubtedly does, of something of the nature of cellulose and containing some of the mineral matters; whilst the pasty substance inside the yeast cells—known as the protoplasm—contains the nitrogenous or protein compounds and fat, together with the larger proportion of the mineral matter.

It will be readily inferred that the elementary matters entering into the composition of yeast must, of necessity, be contained in some form in liquids designed for the production of alcohol by fermentation accompanied by a corresponding increase of yeast, and they are contained in liquids such as grape juice, and in artificial saccharine fluids such as brewers' and distillers' worts, the fermentation of which is a familiar phenomenon.

In order that we may proceed to the study of the yeast cell as a complete and separate organism, and become acquainted with the appearance of the cell at all stages of its growth, some knowledge of the use of the microscope is absolutely necessary, and a few words about the kind of instrument suitable for such a purpose and the way in which it is employed will not be out of place.

What is generally known as the continental model—the chief feature of which is its short foot and tube—is the most convenient

pattern for general use. Very excellent instruments on this plan are now to be had from the various English makers of repute. It is most desirable that the microscope should have stage movements of the ordinary rectangular kind, or better still if it be fitted with Messrs. Swift and Sons' patent movement with the convenient little friction-clutch wheels for holding the glass slip[1]. A good substage achromatic condenser is an indispensable adjunct to secure the best possible performance of the high-power objective, and the lenses must be of undeniably good quality, especially the high-power combination. For all practical purposes, two objectives suffice, viz. a 1 inch and a good $\frac{1}{3}$ inch or $\frac{1}{8}$ inch, which with an *A* and a *B* eyepiece should respectively give magnifications of about 40 diameters and 350 diameters. The low-power combination is useful for work in which a general view is to be taken of moderately minute objects or a special view of a larger object or its parts, whilst the high-power combination serves for microscopic organisms of the nature of yeast or bacteria. A stage bull's-eye condenser is occasionally useful, as also is a camera lucida or a neutral tint reflector for drawing objects or sketching outlines of them, to be filled in by freehand drawing. As a source of light, diffused daylight such as that obtainable from a north aspect is far preferable to direct sunlight; whilst for evening work or when the amount of daylight is inadequate, a good paraffin lamp answers very fairly; but far better is the light from an incandescent mantle-burner or from an electric glow-lamp. In any case where artificial light is employed it is advisable to screen the light on all sides except that from which it proceeds to the mirror of the microscope, or to the bull's-eye condenser, as the case may be.

The qualities of a good microscope may be summed up thus:—

1. Freedom from chromatic and spherical aberration.
2. Good definition and penetration.
3. Flatness of field.

As regards the first requirement, colour rings or fringes round the object represent the worst kind of chromatic aberration: there is also another form of the latter—but not so serious a defect—and that is when the whole field is slightly tinted with colour from over or under correction of the lenses of the objective. Another possible fault of objectives is a distortion of the image, termed 'spherical aberration.' The best test to apply to an objective, to discover whether or not it is

[1] Vide frontispiece of *The Microscope in the Brewery and Malthouse*, Matthews and Lott.

free from this fault, is to use a test-slide ruled in minute squares. If these do not appear as exact squares under the microscope, i.e. if their sides bulge either out or in, then there is spherical aberration.

The second requirement hardly needs any comment, excepting perhaps to say that spherically shaped substances should not look like flat disks, but should show their sphericity plainly.

As regards the third matter—flatness of field—many objectives give very fair results in the central portion of the field of view, whilst objects in the same plane are hopelessly out of focus at the periphery. In such a case an unusual contraction of the field of view by diaphragms sometimes gives a result apparently but not actually better, for it merely excludes the peripheral objects which are more or less out of focus.

Unless the student has had some experience, it is better in purchasing a microscope to avail oneself of the good offices of some one who knows what the needs of the case are, for specially good definition is required for yeast and the organisms that often accompany it, and a microscope that will show these really well is likely to give satisfaction when applied to the examination of any other objects whatsoever.

As regards auxiliary apparatus, the following articles may be mentioned as requisites:—

One dozen or so of glass slips, usual size 3 in. × 1 in.

Cover-glasses, ½ in. circles of thin but not very thin glass.

Three or four stirring rods, 4 to 6 in. long, made of $\frac{3}{16}$ in. glass rod.

A few pipettes or dropping tubes, 6 to 8 in. long, made of $\frac{3}{16}$ in. glass tube.

A small laboratory wash-bottle for distilled or clear spring or filtered water.

A few small stoppered bottles for reagents, dyes, &c.

Some yeast may now be prepared for observation as follows:— A clean glass slip is taken, and with the wash-bottle, or a pipette, a small drop of clear water is placed in the centre. The sample of yeast (it may be from a brewery or distillery, or merely baker's yeast made into a paste with water) is now thoroughly stirred with a pointed glass rod, which before withdrawal may be rotated in the yeast and then against the sides of the vessel in order to remove the excess of adherent yeast. The rod is then carefully dipped into the drop of water on the slip, till a quantity of yeast is int oduced sufficient to impart a slight milkiness when the drop is stirred with the clean end

of the glass rod. A cover-glass is now put on and gently pressed down with the finger-nail, any liquid pressed out at the edge being removed by a small piece of blotting-paper. The yeast so prepared may be first put under the low-power combination (*A* eyepiece and 1 inch obj.); this will show the yeast as tiny spherules, and will give one a very distinct impression of the minuteness of the organism that is being dealt with[1].

If the appliances of the microscope include a *B* eyepiece this may

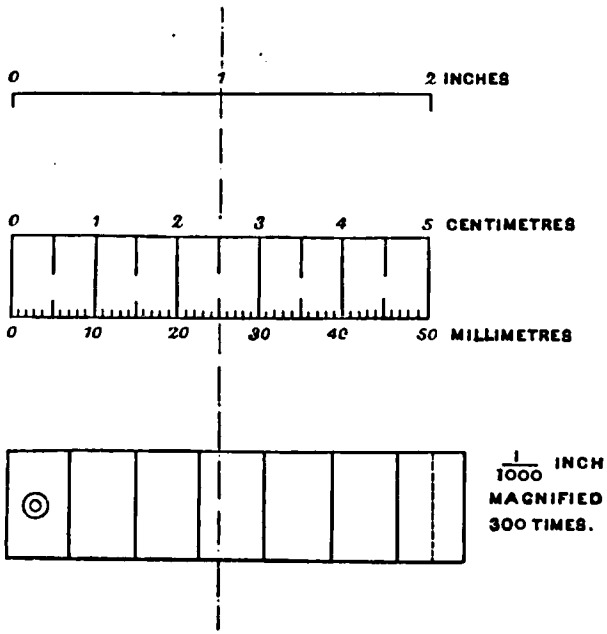


FIG. 1.

be now tried in place of the *A* and a somewhat higher degree of magnification be secured. Finally the high-power objective may be

[1] The following details as to the average dimensions of yeast cells will be of interest at this point :—

Diameter . . .	0·001 mm.
Surface . . .	0·0003 sq. mm.
Volume . . .	0·0000005 cub. mm.
Weight . . .	0·0000000005 gram.

The dry matter in ordinary liquid yeast = 17 per cent.

An ounce avoir. of pressed yeast would contain about 50,000 millions of cells.

FIG. 2

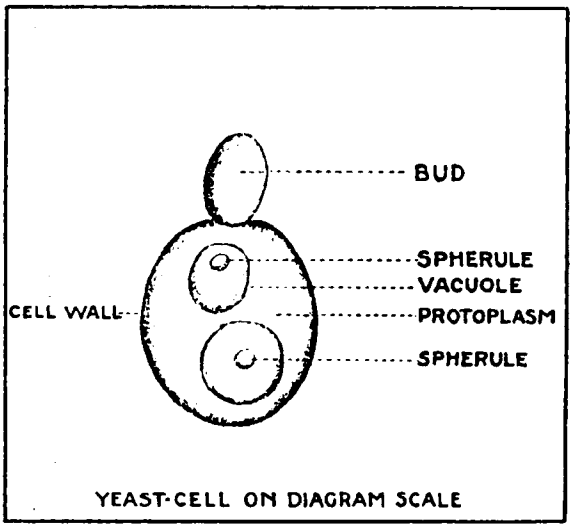
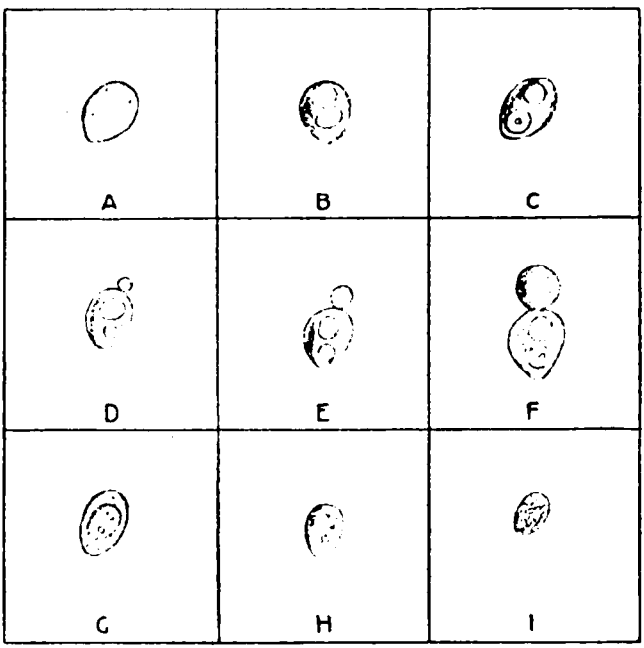


FIG. 3



CONSECUTIVE APPEARANCES DURING GROWTH OF YEAST CELL

$\times \frac{750}{1}$

used, first with the *A* eyepiece then with the *B*, so that the yeast is examined under magnification of some 300–350 diameters (see Fig. 1), when, if the lenses be of really good quality, practically everything in connexion with the cell that is discernible by simple observation will be rendered visible. As a simple matter of manipulation, it is convenient in examining different fields of the specimen of yeast to use the fine adjustment with the left hand, whilst the fingers of the right hand control the stage movements, or, if these last be absent, the glass slip itself. With the higher degree of magnification we find that we are looking at a collection of cells of spherical or ovate form. These cells consist of a sac or bag (Fig. 2)—the envelope or cell wall—containing a pasty or viscid fluid known as the protoplasm. The cell wall, though comparatively thin, has much elasticity and power of resistance: it may, nevertheless, be burst by a sudden shock such as a smart tap upon the cover-glass with the unsharpened end of a lead pencil. If this be successfully done the protoplasm in a diffused granular state is seen to have been forced out from the sac, whose outlines can now be pretty plainly discerned.

On examining perfect cells more closely, we become aware of differences in the nature of the protoplasmic contents, which in some parts of the cell will be seen to be decidedly clearer than it is in others. The clear portions—usually of a spherical shape—are called *vacuoles*, and they are in a measure indicative of a certain stage of activity during the life-history of the organism. They are not visible in very new cells, but become gradually apparent during the maturing of such cells, even in a resting state. In many cases they are probably produced in a very marked way by the withdrawal of nourishment from the dense protoplasm during the reproductive process, and there is no doubt that the vacuoles are also influenced by conditions of temperature and aeration. Whatever be the determining cause, the presence of the vacuole probably denotes that denser protoplasm has been replaced by thinner and more transparent cell-juice, as Reess terms it, a liquid of a more aqueous nature than the rest of the protoplasm.

Healthy cells usually show at least one vacuole, but often two and sometimes three. Inside the vacuole granules or spherules may frequently be seen, sometimes quite small, at other times of very considerable size. These granules or spherules are, not unfrequently, seen to be moving in the clear protoplasm, though the movement is probably not an exhibition of vitality, but of the kind termed 'Brownian,' such as is shown by particles and globules of oil or

resin: possibly the spherules are merely being carried about by currents in the protoplasm itself. Formerly the spherules were regarded as nuclei or central points of vitality, but they are now believed to be simply globules of oily or fatty matter. Yeasts from certain breweries —notably those where what is called the 'Stone square' system of fermentation is pursued—show these spherules very distinctly. London yeast shows them fairly plainly, Burton yeast rather less so and sometimes it does not show them at all. (Plate I, facing page 18.)

On carefully examining full-sized well-vacuoled cells, it is possible to pick out one here and there showing a dark spot, which on causing the cell to move axially by pressing or moving the cover-glass is seen to be located on the cell wall. This spot is the beginning of the bud or young cell, which, as it draws its needed nourishment from the parent cell, gradually enlarges till it attains about the same size as the cell from which it sprang. The area of attachment at the same time decreases, owing to the constriction and separation of the membrane at the base of the cell formed from the bud, till at length the slightest strain suffices to detach the new cell from the parent; and the former goes forth—as it were—to seek its own means of subsistence, whilst the parent cell is left either to bud again or to enter upon a process of decadence. It may, however, happen that where the production of new cells proceeds under very quiescent conditions the new cells remain attached to the parent cell, and chains of four or five cells are then not uncommon. This process of budding goes on in every fermentation where the original yeast was not present in excess, and the different stages may be observed with the microscope. Pasteur, by continuous observation of a budding cell, found that at the end of two hours eight cells had been produced, including the original cell and its first offspring. Such a rate of increase indicates that thousands of cells might be produced from a single budding cell in a very moderate time: it should be said though, that this huge rate of multiplication in a short time is only possible where the original number of cells is small in relation to the volume of liquid to be fermented, and the temperature such as to favour very rapid fermentation. The activity of the yeast cell as displayed in fermentation is—apart from budding—not accompanied by any visible signs of a marked kind under the microscope; to put it into other words, one cannot see the manufacture of alcohol and carbonic acid going on. The vacuoles and spherules of the cell do not seem to perform any traceable functions, but as we said before their appear-

ance is, to a certain extent, indicative of the age and degree of activity of the cell.

To provide yeast cells of different ages for observation under the microscope, a small quantity of readily fermentable liquid such as raisin wort, grape juice, or malt wort may be taken, set with some active yeast, and allowed to ferment in a convenient place where the temperature is at 65°–70° F. As the fermentation progresses, a covering of yeast will most probably be formed, which may be skimmed off from time to time, and the successive skimmings examined microscopically. If some of the yeast be taken which is thrown up last of all from a fermentation which has permitted new yeast to be produced in plenty, it will be found that the cells last removed are the new cells, and that under the microscope they exhibit the following characteristics. They are homogeneous and possess a uniform semi-transparency, and as a rule show no vacuoles or spherules: if they show vacuoles these are but faintly defined, and it will be noticed that there is a general absence of buds. Such new cells cannot be considered as having arrived at their full degree of activity: they are capable of maturing, a process which can be traced from the point we have just spoken of. The different phases are represented in Fig. 3 in consecutive order, starting with the new cell *a*. If the new cells have been produced by fermentation on the practical scale in a brewery, and the yeast has been separated and enters on a period of rest, the lapse of twenty-four to forty-eight hours suffices to produce the matured condition *b* and *c* in which the vacuoles can be discerned and possibly some spherules have made their appearance. In the course of brewing operations the yeast presenting the appearance *c* might be very well employed in starting a fresh fermentation; if so, the commencement of its activity as a ferment would be exhibited by the formation of buds (*d*), and each budding cell would follow the successive stages of development shown in *d*, *e*, *f*, till a perfect new cell was produced, presenting the appearance with which we are now acquainted. The parent cell, if its life-work be accomplished, enters on a period of decadence which is marked by one or two unmistakable features of change, viz. an increase in size of the vacuole (*g*, Fig. 3) and a thickening of the cell wall, probably more apparent than real—also the appearance of more spherules and granulations of the protoplasm. Shrinkage of the cell from the loss of dense protoplasm sets in (*h*), accompanied by granulation and separation of the remaining protoplasm, and the last stage of the deterioration results

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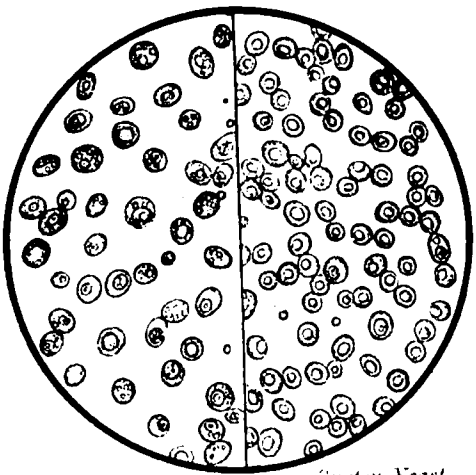
in a shrunken granulated cell (*i*) which not unfrequently presents a slightly yellow or greenish-yellow tint, the original cell being colourless. The final diminution of the cell is to about one-half or two-thirds its original diameter; at this stage it is probably dead, but it is difficult to indicate a precise point at which the cell is incapable of revival, for there are conditions under which the bulk of the yeast in a liquid may appear to be dead, and yet the cells are only torpid and may begin to grow and give rise to active fermentation in a stimulating liquid. For example, the yeast cells forming the deposit of an old bottled beer are often susceptible of revival if brought into a fresh fermentable solution. It will thus be gathered that there is a decadence of the cell which is natural and betokens old age, whilst on the other hand an appearance of decay may be brought about by conditions which are unfavourable to the normal vitality of the cell, and that when these conditions are replaced by favourable ones, the cells are restored again if the inhibitive process has not been going on too long. For all practical purposes, however, we may regard the small granulated cells as useless in any sample of yeast destined to inaugurate fresh fermentation; unless it has proceeded from an ale of exceptionally high specific gravity. A good method of distinguishing living from dead cells, or from torpid or granulated ones, is to add a little colouring matter to the specimen. This is most conveniently done by preparing some yeast on a glass slip, and stirring into the drop a very small quantity of a weak solution of an aniline dye, such as methyl violet or magenta. The cover-glass is put on, and any excess of liquid is sucked up by blotting-paper. Living, active cells resist the stain, if it be used weak enough, but the granulated cells absorb it freely, and can be easily distinguished, under the microscope, from those that are unstained.

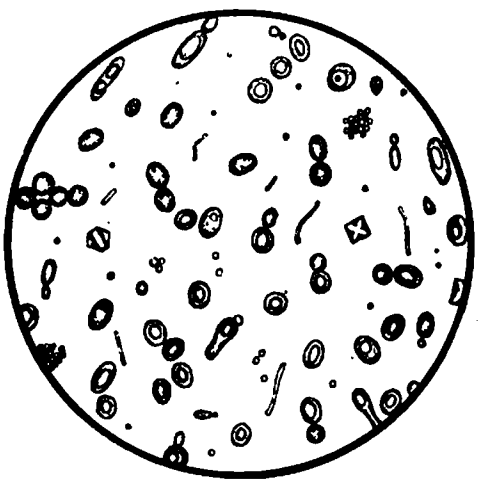
Hitherto we have been concerned with one mode of reproduction of the yeast cell, namely by budding or germination—the ordinary method in fermentative operations; but there is another method which is not only interesting in itself, but is of importance as conveying a clearer idea as to the position that the yeast cell occupies amongst living organisms generally. It has been suspected by many of the experimenters with yeast that there is a close kinship between the fermentative organisms and the moulds, or, to give them their scientific appellations, the Saccharomycetes and the Ascomycetes, both branches of the great family of Fungi; and repeated efforts have been made to establish the conjectured relationship in the following ways:—

Plate I.



London Yeast. *Burton Yeast.*

x 300.



Deteriorated Yeast.

x 300.

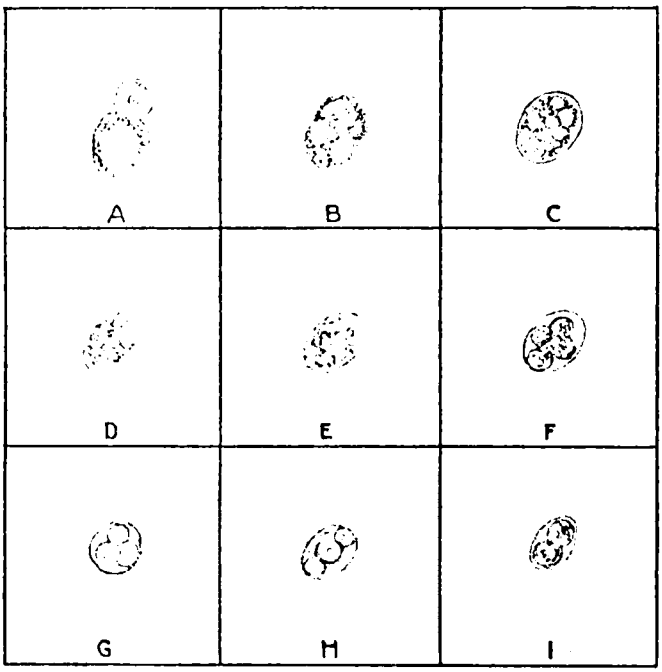
1. By converting the simple cell growth of some Saccharomyces into what is called a mycelial condition, i.e. into a long thread or elongated cell growth.
2. By endeavouring to ascertain if moulds could be made to yield ferment cells by altering the conditions of growth.
3. By seeking to discover if yeast would show any alteration in its mode of reproduction resembling the reproductive processes of the moulds.

Taking the first case into consideration, although the shape of the cell can under certain conditions be very considerably modified as regards elongation (especially in what are known as film growths), it can hardly be said that a truly mycelial condition has been obtained, and the longest cells which have ever been obtained from a growth of Saccharomyces under peculiar conditions, bear but a very distant resemblance to the submerged mycelium of practically any mould. The second case is somewhat different. Several moulds, which naturally grow in free contact with air on the moist surfaces they affect, change their habit of growth when submerged in fermentable liquids such as grape juice or malt wort, and give rise to cells which, in so far as they are able to produce alcohol and carbonic acid, are unquestionably ferment cells but not, properly speaking, Saccharomycetes. *Mucor racemosus*, *Dematium pullulans* and *Fusarium hordei* are examples. In the case of the first and third the ferment cells are so immense in relation to the ordinary yeast cell that there is no likelihood of their being mistaken for the latter, but in the case of *Dematium pullulans* the ferment cells are of such a size and shape that they might easily be mistaken for yeast. Up to the present time, however, no mould has been found to give rise to cells which are unmistakably those of Saccharomyces Cerevisiae.

Reess, a pupil of the eminent fungologist De Bary, paid a good deal of attention to this particular point. By a process of combined reasoning and experimenting he came to the conclusion that ordinary yeast was a composite substance containing not a few species, several of which he succeeded in satisfactorily identifying; after which he took up the general question as to whether these species of yeast could be made to fructify or reproduce as the moulds do. To effect this he altered the conditions under which the yeast was grown, or under which its vitality was maintained, in the following manner. Yeast just removed from a healthy and vigorous fermentation was diffused in pure water and then allowed to deposit, the creamy mass so obtained

was spread evenly upon slices of vegetables (potatoes, carrots, &c.) and left in a moist atmosphere, protected from dust by a bell-glass. If an experiment be conducted on these lines some of the cells sprout or bud in the course of the next day or two, but within the same period a change commences in many of the remaining cells, giving rise to the following appearances under the microscope. The granular contents of the cell show a tendency to become dense and the vacuoles disappear. Some of the cells swell up. Granulations of a more marked character appear in the viscid protoplasm, and round these the finer granulations collect and form two to four islands each, with a dense granule as nucleus. In another twelve to twenty-four hours, and some six days after the commencement of the experiment, the granular groupings become respectively enveloped by a tough membrane. This membrane becomes thicker, and the outline of each new cell or spore thus produced becomes clearer, whilst the original cell envelope is stretched over the whole, and, besides enclosing the newly formed spores, also surrounds any residual protoplasm. The changes are shown in Fig. 4, *a*, *b*, *c*, *d*, *e*, *f*. The original cell wall may now give way at any time by simple pressure from inside or by partial dissolution and consequent weakening, and the spores are then liberated in twos, threes, or fours, according to the way in which they have been formed (Fig. 4, *g*, *h*, *i*). The bursting of the spore-containing cells is accelerated by bringing them into a fermentable liquid when the spores begin to bud and the pressure increases rapidly. If the newly separated spores be introduced into a similar liquid budding also takes place, and this results directly in the formation of fresh yeast cells, see Fig. 5; 1 and 2 show the budding of two triad groups of spores, and 3 of two tetrad groups. Now this method of reproduction by the formation of spores or ascospores (also known as endogenous division) is akin to a well-known method amongst the moulds, viz. the formation of an ascus containing spores, and this fact led Reess and De Bary to express the belief that yeasts are genetically associated with the moulds and so may be regarded as greatly reduced or deteriorated Ascomycetes, or lower forms in the evolutionary process by which moulds have been developed. It is nevertheless convenient to draw a line of demarcation between the ferments proper and the moulds, and to consider that the Saccharomycetes form a distinct genus, though unquestionably possessing characteristics which tend to link them with the moulds.

FIG. 4



SPORULATION OF YEAST

AFTER REESS)

Rcess's observations, published in 1870, in connexion with the sporulation of yeast have since been amply substantiated. They were repeated within a very short time by Engel, who found that if moistened plaster of Paris was substituted for the slices of vegetable a more perfect sporulation was obtained. Hansen, the great Danish investigator, has devoted a great amount of attention to the sporulation of different varieties and species of yeast, and has, as we shall see, turned his observations to much account as a means of research into the characteristics of the genus Saccharomyces; indeed, we may say at this point that sporulation is now rather generally regarded as one of the proper attributes of the Saccharomycetes, and therefore the designation is restricted to the alcoholic ferments which are able to reproduce themselves in this manner.

One of the interesting questions connected with the yeast organism is as to whether the cells ever visibly contain or can be made to exhibit a true nucleus, that is to say a point or spot or aggregation which can be proved to be the starting-point of reproductive changes, or which participates actively in the latter. We have already seen that the term nucleus has been applied to the granules or spherules found in the vacuoles of yeast cells, but there is no reason to suppose that they play any important part in the production of new cells; they are, in all probability, either fatty matter or glycogen. Many investigators have tried to identify a nucleus in yeast cells, and Hansen has found a plainly visible nucleus in film-forming yeasts. Rather recently Richard Wager[1] has published a very interesting research on this subject, the following being his main conclusions:—All yeasts possess a nucleus and nucleolus, the latter in young cells being in intimate contact with a vacuole. The nucleolus is always met with, and appears to be a perfectly homogeneous body which sometimes has a granular appearance owing to the presence of granules in proximity to it. During the budding the nucleus shows no markings during its division, which last may be regarded as a direct splitting into two parts, the division taking place either in the junction between the cell and its bud or—more rarely—in the mother cell, one part passing into the daughter cell.

In face of this discovery by Wager it is reasonable to assume, although he does not say so, that the nucleus is immediately concerned

[1] *Annals of Botany*, 1898, p. 499; also *Trans. Fed. Insts. of Brewing*, 1899, No. 5.

when the act of sporulation takes place, and that a definite partition occurs in this case also, the number of portions into which the nucleus divides determining, in all probability, the number of spores formed in each cell.

Experiments that may be performed in connexion with Chaps. I and II.

1. Examination under the microscope of some purchased baker's yeast or pressed yeast, diffused in water as described.

2. Examination in the same manner of a specimen of brewer's yeast in the ordinary pasty or creamy condition.

3. Stain one or other of the above preparations with a solution of methyl violet, magenta, or eosine (see Appendix).

4. Obtain some grapes showing a good 'bloom' and with a camel's-hair brush and some distilled water paint off the bloom into a few drops of water till a decided opalescence or turbidity ensues, then examine the liquid under the microscope. Ferment cells or Saccharomyces will be found, together with other organisms and various fortuitous matters, e.g. spores of mould, bacteria, silicious particles, &c.

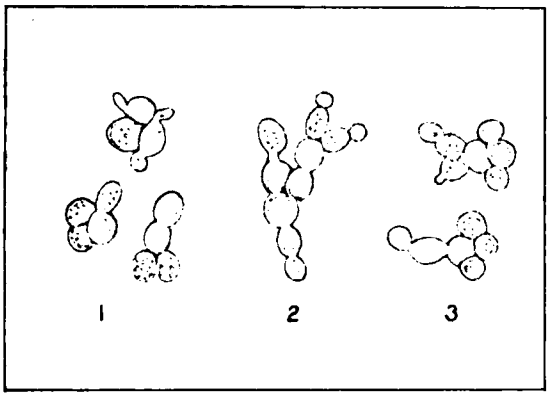
5. A similar experiment may be performed with a handful of barley, which may be wetted with an ounce or two of water in a small basin and worked with the fingers so as to detach adherent dust. The washings poured off and allowed to settle will yield a deposit fairly rich in organisms, amongst which yeast cells may often be distinguished.

6. Prepare a strong wort by infusing about ½ lb. of chopped raisins with 1 pint of boiling water. Strain through muslin or calico and filter bright through filter paper. Add a teaspoonful of fairly active yeast and put in a warm place (temp. between 65° and 70° F.). Active fermentation will set in. Skim off the yeast as it rises and note its appearance at different intervals; the newest cells are usually those that come to the surface at the close of the fermentation.

7. Some of the washings obtained in 4 and 5 may be set in some bright raisin wort and will in all probability originate fermentation.

8. An experiment may be made to obtain spores. Use the yeast obtained from experiment 6; wash and put on disks of potato or of plaster of Paris; set in some water on a plate. Cover with a bell-glass and put in a fairly warm place, 65° to 70° F. Examine every day up to eight or ten days.

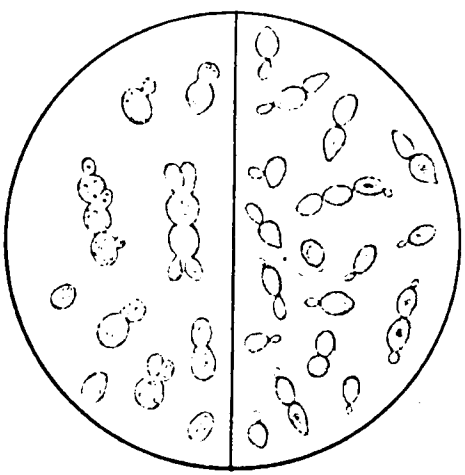
FIG. 5



BUDDING YEAST SPORES

(AFTER REESS)

FIG. 6



'LOW' FERMENTATION YEAST

TWO VARIETIES

CHAPTER III

THE SACCHAROMYCETES, AND OTHER ORGANISMS ACTING AS ALCOHOLIC FERMENTS

IN the preceding chapters we have taken into consideration the general phenomena of alcoholic fermentation and associated them with a specific organism; and have, moreover, traced the life-history and reproductive processes typical of this class of organism. We may now turn our attention to the genus Saccharomyces as a whole, and inquire as to the species and varieties which have been satisfactorily identified, and the means that have been employed to this end. A microscopical examination of yeast taken from liquids—fruit juices, &c.—which have entered into spontaneous or fortuitous fermentation, very quickly reveals the fact that the ferment cells show a considerable diversity in form, a diversity which stands in marked contrast to the regularity in size and shape presented by a good specimen of baker's or brewer's yeast. A few experiences of this kind would rather lead one to infer that the variation in form was not merely due to possible differences in the nature of the liquids from which the cells were taken, but that the cells originating the fermentation were themselves diverse in form. This inference might be brought to the point of proof by examining the cells attached to the fruits, the juices of which had been allowed to ferment, and noting the persistence of particular forms of cells in the fermentations; but it could be even more clearly established by setting different portions of the same liquid—say a fruit juice—with yeast from different sources. Other essential differences between the products of different fermentations can be detected by the senses, such for instance as the flavour, the odour, and in some cases the appearance, and this may hold good even of the same kind of liquid when fermented by yeast from different sources.

At the period of Pasteur's and Reess's investigations (1870) two methods of distinction were mainly relied upon: (1) the use of the microscope to display such differences of form as might exist between

the cells of different species—scientifically termed 'the morphological differences'; (2) the physical and chemical examination of the fermenting or fermented liquid in order to detect variations in appearance, taste, and smell, as compared with the results obtained by Saccharomyces Cerevisiae, or ordinary beer yeast.

Imperfect as such methods were, they nevertheless proved adequate for the distinguishing of the following species, which, although susceptible of further differentiation into varieties, may conveniently be taken as typical of alcoholic ferments of general occurrence :—

Saccharomyces Cerevisiae of 'high' and 'low' fermentation.
„ Pastorianus.
„ Ellipsoideus.
„ Coagulatus (caseous yeast).
„ Exiguus.
„ Apiculatus.
„ Minor.
„ Conglomeratus.

A short description of each of these in turn :—

Saccharomyces Cerevisiae, or brewer's yeast, in the form in which it now exists, is the result of what may be regarded as an educational process applied to yeast originally encountered or occurring in a wild or uncultivated state. Two distinct methods of brewing have given rise to two well-defined varieties of Saccharomyces Cerevisiae, and centuries of use have brought it to what it is in its two chief phases—a ferment of considerable uniformity in appearance and action. One main variety of S. Cerevisiae is associated with the normal brewing process of the United Kingdom, and is generally known as 'high' yeast, reference being made thereby to the temperature at which the fermentations are conducted. The other main variety is employed in the process of lager-beer brewing, and since the accustomed temperature of the fermentations is well below the mean temperature of the air, and the yeast does not come to the top of the liquid, but works largely at the bottom of it, this kind of Saccharomyces Cerevisiae is frequently spoken of as 'low' or 'bottom' yeast. It is not only largely employed on the Continent, but also in the United States.

It will be readily understood that in aiming to secure the most favourable conditions for their respective methods, brewers have been —not altogether consciously—working towards the production of two distinct types of yeast, fitted to carry out 'high' and 'low' fermentation respectively. Now the yeasts of the two processes are not industrially

interchangeable, and it is a nice question whether they have proceeded from distinctly different varieties—or even species—of S. Cerevisiae, or are the modified forms of a common progenitor. It is true that, broadly speaking, high-fermentation yeast fails to carry on perceptible fermentation at the low temperatures employed in the production of lager beer, and vice versa, but there are some interesting—if not modifying—points connected with this consideration. Reess succeeded in getting 'low' yeast to bud at temperatures 9° to 18° F. higher than those employed in lager-beer brewing, and S. Cerevisiae of 'high' fermentation, set at 40° to 43° F. (the temperature of low fermentation), vegetated after the lapse of six days in typical low-yeast forms; hence Reess considered that the 'high' and 'low' ferments might be modifications of the same species. Hansen has succeeded in getting 'low' yeast to ferment at higher ranges of temperature, but he found that it immediately reverted to its former mode of action when submitted to low-temperature fermentation. The author has also found that it is not difficult in some cases to get low-temperature yeast to ferment at higher ranges of temperature, and even to function as baker's yeast, but the results of the fermentation are, however, as regards the flavour of the fermented liquid, not the same as if the accustomed low temperature of fermentation had been employed.

For our present purpose we may regard the large proportion of cells in a clean and active brewer's yeast as being those of Saccharomyces Cerevisiae. A microscopical examination of the yeast of high fermentation gives us the following information—which, with some slight reservations may be considered as applying to the yeast from 'low' fermentation.

The average diameter of the cells of ordinary brewer's yeast is about $\frac{1}{3000}$ inch, or 8 microns[1] (see Fig. 1).

The microscopical characteristics which, as a rule, denote an active and healthy yeast are the following:—Uniformity of size and shape; clearness of the cell outline, indicating a strong cell wall; presence of vacuoles, which should show clearly and be of fair size—neither large nor small; for if the vacuoles are barely perceptible the yeast is probably too young for use, and if the vacuoles are large it is a sign of exhaustion. The granules or spherules should not be too plainly

[1] The Greek letter μ stands for micron, the agreed term for $\frac{1}{1000}$ of a millimetre, which is taken as the microscopical standard of measurement on the metric system. $\frac{1}{1000}$ inch = 25·4 μ.

discerned, though some yeasts naturally show them very plainly, and due allowance must be made for this fact. There should be no noticeable proportion of dead or granulated cells or foreign matter, such as strange yeasts, filamentous bodies, 'grounds,' or dirt of any kind. In Plate I are shown healthy, active specimens of Saccharomyces Cerevisiae, also a specimen of yeast in an exhausted state accompanied by extraneous matters.

Considerable differences may be detected microscopically between yeasts arising from the different methods of fermentation pursued in the United Kingdom :—

London yeast (see Plate I) :—Cells rather larger than the average size for brewer's yeast; rather irregular in size. General tendency to a spherical shape, but also bluntly ovate. Vacuoles large. Granules or spherules rather marked.

Burton yeast (Plate I) :—Average-sized cells. Uniform size and shape. Vacuoles well defined, but not very large; very often two vacuoles visible, a large and a small one. Great tendency to an ovate form, sometimes almost pointed at one end.

Yorkshire stone-square yeast, also yeasts from slow-skimming processes (e.g. some Scotch yeasts) :—Rather irregular in size and shape. Rather above average as regards size. Tendency to spherical shape. Vacuoles distinct. Spherules large and distinct, sometimes more than one in the same cell or even vacuole; occasionally seen to be moving about.

The cells of Saccharomyces Cerevisiae produced by these high-fermentation methods may often be seen in the budding state, but seldom in the branching condition, that is to say, with three or four cells adherent and forming a chain, a condition which is said to be very common in low-fermentation yeasts. Low-temperature fermentation is of such a slow and quiescent nature that the newly formed cells tend to remain where they are produced, and remain for a longer time adherent to their progenitors. Fig. 6 shows this peculiarity, which seems to be the chief distinguishing feature of S. Cerevisiae of low fermentation, or, as it is sometimes termed from its mode of working, 'bottom' yeast, the cognate term for the English ferment being 'top' yeast.

We may now direct our attention to the other species of yeast before mentioned, which, speaking generally, present very different features in comparison with S. Cerevisiae. So marked are the differences, that if the growths of these other species be approximately

pure there is not much difficulty in satisfactorily identifying them; such a state of purity as this is, however, seldom encountered, unless special methods have been employed to secure it.

Saccharomyces Pastorianus.

This alcoholic ferment was—as the name implies—identified by Pasteur, and was subjected to a critical examination by Reess, and afterwards by Hansen who has succeeded in separating and identifying several varieties of this species. The typical ferment was discovered in wines undergoing the secondary or maturing fermentation; it is also very frequently found in beers undergoing secondary fermentation, and may be said to be of comparatively common occurrence. There is little doubt that it can easily ferment grape sugar, but its behaviour as regards cane sugar is doubtful. The peculiar feature of this species is the elongated and somewhat club-shaped cell of rather variable size (see Plate II). The cells usually bud at the broader end and a little towards one side, thus presenting a characteristic appearance which is rather easily recognized, and enables one to pick out this ferment in a mixture of yeasts more readily than most other kinds. The cells may, under ordinary conditions, attain the length of 18 to 22 μ, with a width of some 4 to 6 μ; but a considerable divergence on either side of these measurements is possible, according to the particular variety of the ferment and the conditions attending growth. S. Pastorianus sporulates somewhat readily. It is a common cause of the secondary fermentation of English beers, and can be frequently found in bottled-beer deposits. When present in beers it may give rise to troublesome 'frets,' and cause peculiarities of flavour which militate against the success of the ale which is so affected.

Saccharomyces Ellipsoideus,

sometimes called Saccharomyces Ellipticus (Plate II), is the wine yeast of Pasteur, or the yeast which is generally present during the vigorous fermentation of grape juice or 'must.' Reess submitted this yeast to a close investigation, and found that it was capable of producing spores under the conditions that are known to favour this mode of reproduction. Saccharomyces Ellipsoideus is distinguished by its uniformly elliptic cells, which in shape somewhat resemble a grain of rice or an ant's egg. These cells average about 6 μ in their longer

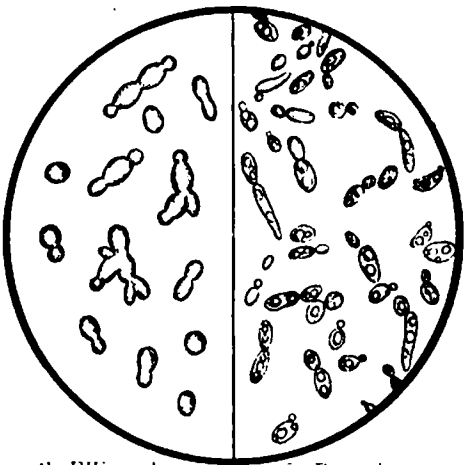
dimension, and 3 to 3·5 μ in the shorter axis. Budding usually takes place at the poles of the longer axis, and frequently both ends of the cell give rise to a bud simultaneously. Reess says that this species of yeast will ferment slowly at a temperature of 5° to 10° C. (41° to 50° F.), and under these conditions the new cells separate readily from the mother cells. At temperatures lying between 15° to 18° C. (59° to 64·5° F.) it is a vigorous ferment, and at this more favouring temperature bands or strings of cells may be formed. Saccharomyces Ellipsoideus, besides growing freely in grape juice, will grow in cane sugar dissolved in yeast water (see Appendix), in grape-sugar solution, and in beer wort. Hansen has identified two varieties of this particular species, S. Ellipsoideus I and S. Ellipsoideus II.

This yeast is not infrequently found in beers which are undergoing secondary fermentation, and when it is the predominating ferment form in this connexion, its presence is often associated with some degree of turbidity and a peculiar odour, constituting what is called a 'sick' state of the beer: sometimes the smell is decidedly unpleasant, when the stronger expression 'stench' is employed to define it. If the beer so affected has been carefully brewed from sound and good materials the complaint usually passes off, and the beer which was at a time undrinkable may mature into a very excellent beverage. Beers brewed from very hard sulphated waters, such as Burton-on-Trent supplies, are probably more liable to this disorder than the beers of other localities, but now that much less Burton ale is brewed for long storage the complaint is less common, for it was in the 'stock' ales that the defect was more often noticed.

Saccharomyces Coagulatus.

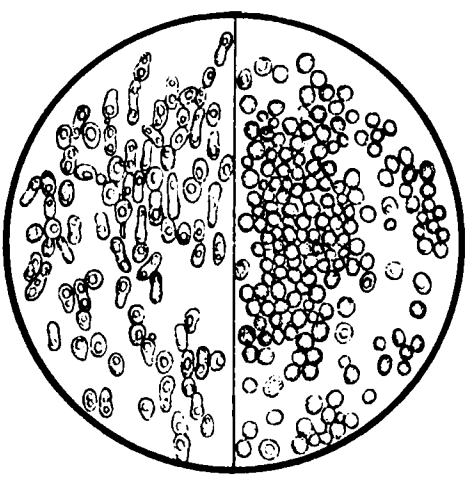
The cells of this species are distinguished by their marked and curious tendency to agglomerate and form a curdy or cheesy mass. So pronounced is this tendency that if a small quantity of this yeast be stirred with a small volume of water and then added to a large bulk of the latter, the yeast falls out quickly in a flocculent state, and can be suspended in water again and separated time after time, and thus washed free from loose cells and extraneous matters. If one has succeeded in securing a fair specimen of this particular yeast it may be kept in a state of comparative purity by successive fermentations, and washing the crop of yeast obtained thereby. No other yeast lends itself so conveniently to this mode of treatment as S. Coagulatus. It has been affirmed that S. Coagulatus represents a state into which

PLATE II



S. Ellipsoideus. (*Reess.*) × 350.

S. Pastorianus. (*M. & L.*) × 300.



S. Coagulatus I.

S. Coagulatus II.

× 300.

various species of yeast may enter under favouring conditions. The writer's experience is that this is not so, for although it is true that some types of S. Cerevisiae may show a slight tendency to coagulate the condition is but transient and may be easily destroyed, whilst the habit of true S. Coagulatus is most persistent.

There are at least two varieties of this alcoholic ferment. The one earlier known was discovered by Pasteur, who described it as a caseous ferment, doubtless from its curdy nature and appearance. He obtained it by exposing a fermentable liquid set with Burton yeast to a temperature of 122° F. for one hour. The other varieties and species of Saccharomycetes being killed at this temperature, S. Coagulatus alone grew, and thus obtained, exhibited cells allied in shape to the typical forms of S. Pastorianus, and so the name of S. Coagulatus Pastorianus[1] has been suggested for this variety of caseous yeast. The same or a closely allied variety is discoverable in bottled beers and ales submitted to what is known as the 'forcing test[2].'

Some years ago the writer described a variety of S. Coagulatus, also obtained from Burton yeast, which was undoubtedly something different to that described by Pasteur. This variety, when in a state of purity, consists mainly of spherical cells of two-thirds to three-fourths the diameter of the cells of ordinary Saccharomyces Cerevisiae. The diameter of the cells is about 5 to 6 μ. The yeast sporulates readily. The variety may be conveniently described as S. Coagulatus II[3]. The two yeasts are shown side by side in the illustration in Plate II.

The writer has obtained S. Coagulatus from various sources of which the following are some:—Red 'spent-hop' ould (Oidium Lupuli) sown into beer wort. Bottled and forced beer deposits. Yeast or torula growing in the air on wet wood surfaces; also from a specimen of lager-beer yeast, which in this particular case seemed to consist mainly of some variety of S. Coagulatus. In this case the 'low' yeast was not only able to function as a 'high' ferment, but made a very excellent

[1] Vide *The Microscope in the Brewery*, &c. (Matthews and Lott), p. 47.

[2] This test consists in filling small flasks of a special pattern with an average specimen of a particular brewing of beer, and putting them on a copper water-tray which is kept at a constant temperature of 78°–80° F. The natural changes in the liquid are greatly accelerated by the high temperature, and a microscopical examination of the sediment after a few weeks, as well as noting the general characteristics of the liquid, gives much information as to the probable history of the sample. The test can also be applied to wines, especially clarets.

[3] Vide *The Microscope in the Brewery*, &c. (Matthews and Lott), p. 47.

baker's yeast in addition, so that the range of temperature, throughout which it was able to display active fermentative powers, was very great indeed.

There is no particular evidence extant as to the behaviour of S. Coagulatus Pastorianus in relation to fermentable solutions, but as regards S. Coagulatus II one can say that it possesses a degree of activity not inferior—under the most favourable conditions—to Saccharomyces Cerevisiae. It is energetic as a ferment at temperatures between 60° and 70° F., and is still more so at higher ranges of temperature. S. Coagulatus can carry the fermentation of a solution to a very advanced point, further in some cases than S. Cerevisiae is able to do, but a somewhat coarse and peculiar flavour results if it be employed in beer wort.

The caseous yeasts—as may be inferred from some of the foregoing facts—may be regarded as of relatively common occurrence. It is not difficult to get a specimen from bottled ales, especially if they are of Burton brewing.

Saccharomyces Exiguus

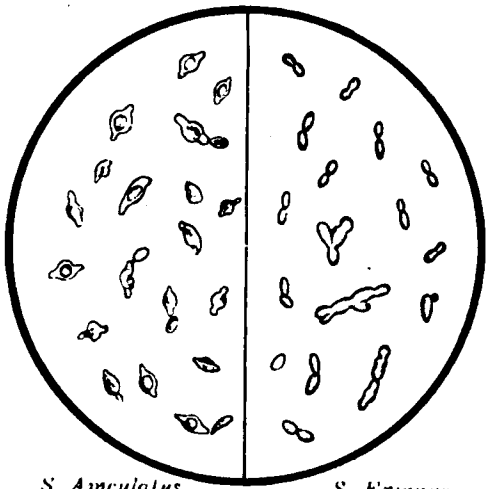
was observed by Reess in fruit juices in a state of fermentation. The cells show—like S. Ellipsoideus—an elliptical shape, but are much smaller than the ferment just named. As described by Reess the cells are about 5 μ long and 2·5 μ in their largest cross diameter, and show but little tendency to branch. It forms spores, and may be classed as a true Saccharomyces. Reess found the ferment also in beers undergoing secondary fermentation.

Matthews and Lott describe a smaller variety of S. Exiguus which they have seen in English beers, in which its presence was giving rise to a rather persistent cloudiness or turbidity, creating thereby a difficulty for the brewer. It has been discerned too in connexion with other abnormal conditions of beer. It is a sluggish ferment in the ordinary fermentable liquids. It has a general tendency to agglomerate into small masses in beers in which finings are in suspension, or it will even bring small portions of the finings up from the 'grounds.' The microscopical appearance of this yeast is shown in Plate III.

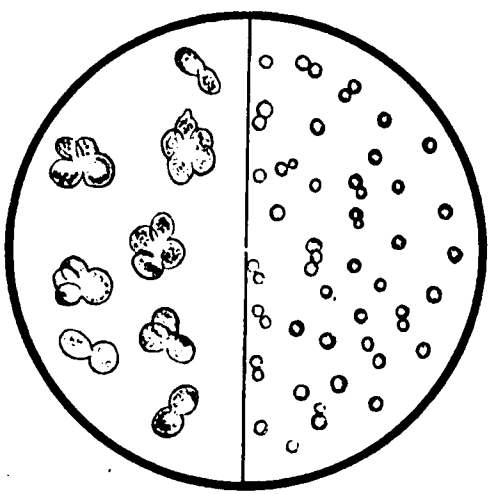
Saccharomyces Apiculatus,

or the 'pointed' yeast, is abundant on sweet fruits during the period of ripening, and occurring as it does on the grape, is often found

PLATE III.



S. Apiculatus. (*Hansen.*) × 350. *S. Exiguus.* (*Reess.*)



S. Conglomeratus. (*Reess.*) × 600. *S. Minor.* × 300.

associated with primary vinous fermentation. It has also been observed in Belgian breweries, where the worts—according to one of the methods of procedure—are allowed to enter into spontaneous fermentation. Foreign beers would seem to contain it not unfrequently, but it is not met with in English beers, although it is able to ferment beer wort feebly. S. Apiculatus was described by both Pasteur and Reess, the latter of whom enters particularly into a description of some of its more striking peculiarities. The mature cells of this ferment are pointed at the ends of the longer axis, and thus resemble a lemon rather nearly in shape: the young cells have rounded ends and rather resemble those of S. Ellipsoideus, but as they mature they acquire the characteristic pointed appearance of the older cells from which they spring. Budding—as is usually the case with the elongated forms of the Saccharomyces—takes place at the ends, or near the ends, of the major axis of the cell. The average size of the cells of S. Apiculatus is from 6 to 8 μ in length, and 2 to 3 μ in width. Reess was unsuccessful in his endeavours to obtain sporulation of this yeast. Engel describes a modified kind of spore-formation, and, recently, Beijerinck has succeeded in obtaining spores under aërial conditions of exposure of the yeast, but not in the moist state on plaster blocks. From the point of view of a rigid classification, it is a little doubtful as to whether S. Apiculatus is a true alcoholic ferment, but one may as well make the observation here that nature furnishes many such examples of the lack of conformation to a cast-iron definition; rather than this there are distinct connecting links between the genera and species, or even families, of living organisms.

S. Apiculatus has been made the subject of a special research by Hansen, and his observations are very valuable and instructive, as tending to throw light upon the behaviour of ferment cells generally. Hansen found that during the winter months the cells of S. Apiculatus were discoverable in the earth underneath the shrubs on which it made its appearance during late summer and autumn. As certain fruits ripened—plums, cherries, &c.—so the ferment was discernible upon them, and the washing effect of rain, or the falling of the ripe fruit to the ground, caused the cells of the ferment to become intermingled with the soil, where they remain and retain their vitality until liberated by the drying up of the soil and its dispersion as dust, when the cells once more find their way on to the trees and shrubs. Hansen found also that the cells could retain vitality even after a three-years' sojourn in the ground. He could not get the yeast

to sporulate under the ordinary conditions. S. Apiculatus appears to ferment the fruit sugars, dextrose, and levulose (fructose), but not cane sugar or maltose (malt sugar). An illustration of the ferment is shown on Plate III.

Saccharomyces Minor

is a small ferment form discovered by Engel in leaven, and supposed by him to be the active agent in the fermentation of flour dough. From the technical point of view it is not of much importance, for it does not figure in fermentation processes. Spontaneous fermentation of flour-paste or dough is but seldom made use of, baker's yeast being so generally employed in bread-making and Saccharomyces Minor is of comparatively rare occurrence in fermenting liquids. The ferment is nevertheless of some scientific interest, and as an example of a marked departure in size from the ordinary forms of Saccharomyces it is peculiarly interesting. At present it is the smallest yeast known, the cells being only about one-half the diameter of those of S. Cerevisiae, and they may be even less than this (see Plate III). Their average diameter may be taken as about 4μ (say $\frac{1}{6000}$ inch). The cells are usually perfect spheres. Since the yeast is able to form spores it may be regarded as a true Saccharomyces. It is sometimes found in beers, but seldom growing at all freely. Strong sugar-syrups, molasses, cereal sugar-syrups, &c., in a state of fermentation sometimes exhibit this ferment, and the writer's observations give rise to the conclusion that S. Minor and S. Coagulatus II are able to ferment saccharine solutions of a degree of concentration that would be inhibitive to most of the other species of Saccharomyces. The presence of S. Minor in a brewery yeast—and the same thing may be said of all the species of Saccharomyces, excluding S. Cerevisiae—would constitute an impurity. Such incursive cells are often spoken of as 'wild' yeast, and the term is a convenient one: there is a tendency, however, to narrow the application of this term to the yeasts other than S. Cerevisiae which are known to produce abnormal results and create difficulty for the brewer, for bad results are not always concurrent with the appearance of strange cells.

Saccharomyces Conglomeratus

is an uncommon and very curious form of Saccharomyces described by Reess, who discovered it in wine fermentations, but came to no precise conclusions as to its functions as a ferment. The great

peculiarity of this ferment, and that which makes it worthy of mention, is that the cells bud at several points simultaneously, and these so close together that the protuberances caused by the enlarged buds are almost, if not quite confluent, and thus an appearance is produced quite dissimilar to any other species of yeast at present known. The diameter of the cells is about 5 to 6 μ, so that it is rather a small yeast. The yeast is shown in Plate III. It is of no technical importance, but is interesting morphologically. Reess succeeded in obtaining sporulation of S. Conglomeratus under the conditions which favoured sporulation in other species of Saccharomyces.

All the foregoing Saccharomycetes may be regarded as species which have in some cases, notably S. Cerevisiae, S. Pastorianus, and S. Ellipsoideus, shown themselves in the hands of skilled investigators to be resolvable into varieties. The question as to the differing functions of these varieties will occupy us at a further point, and so it is not necessary to dwell on this matter, interesting as it unquestionably is, but rather to turn our attention to a few organisms which are capable of exciting alcoholic fermentation, but which are not regarded as belonging to the family of Saccharomyces. The following examples may be given :—

Mycoderma Vini.

A rather commonly occurring organism which forms a white or yellowish-white greasy film on beer that is left exposed to the air in shallow vessels, also around the taps and bungholes of casks. Thus found it is absorbing oxygen freely from the air and destroying the alcohol of the beer, forming from it carbonic anhydride and water. Its growth under these conditions is usually fast and sometimes very rapid. Engel calculated that in forty-eight hours a single cell of Mycoderma Vini may produce 38,000. The organism consists of elongated cells budding at the extremities, bearing a strong general likeness to S. Pastorianus, and at times almost impossible to be distinguished from this true Saccharomyces. The dimensions of the cells are somewhat variable, according to the conditions attending growth. The length may be taken as about 7 to 9 μ by 2 to 3 μ broad. (Plate IV.)

Pasteur found that when the cells of Mycoderma Vini are submerged in a fermentable liquid, it is able to act as a slow alcoholic ferment, carbonic anhydride being liberated at the same time that alcohol is formed. The cells meanwhile undergo some change in appearance, filling out and becoming brighter and more highly

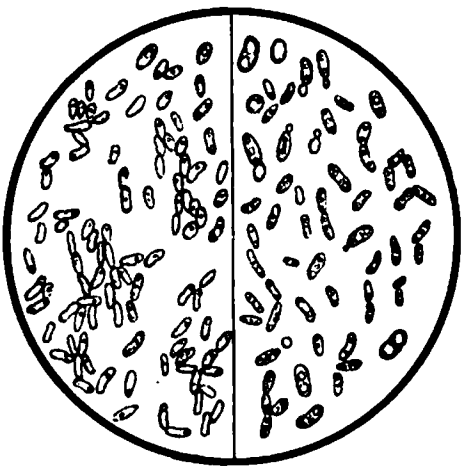
vacuoled. This kind of growth, interesting as it unquestionably is—and instructive too as regards submerged growths—can, however, only be regarded as abnormal, and the organism reverts most readily to the aerobian state if the liquid containing the submerged form be freely exposed to the air.

Pasteur has shown that some of the true alcoholic ferments may pass from the ordinary submerged mode of growth—scientifically termed the anaerobic condition—to a growth on the surface of the liquid which contains them. Such a growth in free contact with air, and indeed promoted by it, is termed the aerobic state. When yeasts do this in a quiescent liquid a film or veil forms on the surface, and may even extend to the walls of the flask. This appearance is described also by the more homely term 'mothering.' Hansen, besides utilizing the conditions under which sporulation takes place as a means of distinguishing between different species and varieties of yeast, has further dealt with the problem by noting the conditions under which films or pellicles are formed by Saccharomyces on the surface of fermentable or fermented liquids. These films generally form towards the close of fermentation carried out experimentally in glass flasks, and have their visible commencement in small islets of yeast which have come to the surface from the sedimentary yeast, and, being specifically lighter than the liquid, remain there and develop the aerobian or film form of the ferment. These growths may be said to retain or exhibit in a very general way the forms from which they sprang; but a very wide departure from the typical form is usually discernible, and the shapes of some of the cells are truly remarkable. When dealing with the subject of pure cultivation of yeast we shall have occasion to dwell more fully on this matter.

Moulds acting as Ferments.

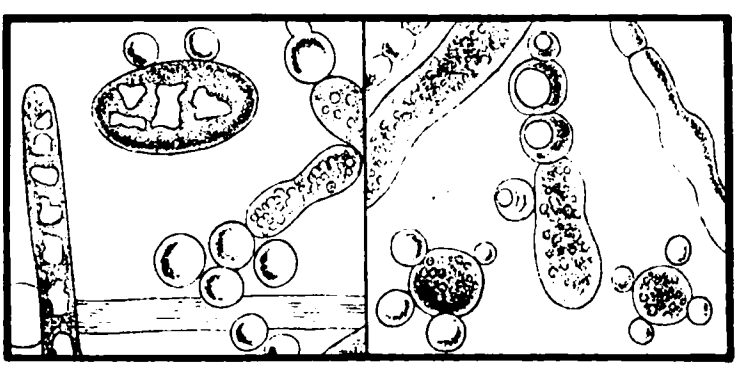
So many of the moulds are capable, when submerged in a fermentable solution, of giving rise to alcoholic fermentation that one cannot regard it as a very extraordinary function: it is a curious one nevertheless, as, indeed, is the production of alcohol by living cells of various kinds. This formation of alcohol is a more widely spread phenomenon than might be supposed, and is calculated to give rise to scientific speculations of no little interest: one would infer, amongst other things, that the lower organisms are not affected by alcohol in the same way as the higher ones, for some of the alcoholic ferments

PLATE IV.



Mycoderma Vini.

Aerobian. × 300. *Submerged.*



Fusarium Hordei. *Mucor Racemosus*

× 300.

can exercise their vital functions in the presence of a high percentage of alcohol, and retain vitality for years in solutions of considerable alcoholic strength, such as strong ales and stouts. To return however to the moulds, some of which are able to display a very pronounced power of exciting alcoholic fermentation. If a mould be set in a suitable saccharine liquid, it may grow in two distinct ways, like Mycoderma Vini; (1) a simple branching or mycelial surface growth, which is simply an extension of its ordinary mode of growth in the air. The commonly occurring mould, Penicillium Glaucum, is a good example of this. Its growth in a saccharine liquid may begin by the formation of floating white islets with a bluish or greenish blue centre: this is a mixed aerobian and submerged growth. (2) As the islet increases in size, the weight of the mass causes it to sink more deeply into the liquid, and a copious submerged growth is the result, which, when examined under the microscope, shows an interlaced mass of tubes. This mass is the submerged mycelium, and it is growing at the expense of the components of the liquid, and, in the case of the mould which is being described, the writer has found that a small amount of alcohol is formed. No separate spherical cells are visible. Another and more advanced mode of growth as an alcoholic ferment is to be seen in the case of the mould Mucor Racemosus, a true Ascomycetes or mould, which forms in its normal aërial growth a definite spore case or ascus. If a specimen of this mould be conveyed into a fermentable solution such as beer wort, a mixed aërial and submerged growth commences, which speedily becomes a submerged growth only. A buff-coloured felted mycelium forms, which may spread through the greater part of the liquid; its upper surface is at or near the surface of the liquid, and bubbles of gas may be seen to be enmeshed in it which occasionally liberate themselves or can be liberated by shaking the containing vessel. Under the microscope huge branching cells of a tubular or cylindrical kind are seen, which as they elongate tend to give rise to spheres, and from these may bud true spheres, which when detached are practically ferment cells, being able to bud freely at several points of their surface. These cells are often quite $2\frac{1}{2}$ times the diameter of an ordinary yeast cell. (Plate IV.)

Mucor Racemosus produces but a small percentage of alcohol, about 4 per cent. by volume; the liberated gas is carbonic anhydride. If the felted submerged mycelium be removed from the liquid in which it has been formed, and exposed to the air on a nutrient surface,

such as bread paste or moist dough, it will once more give rise to the aërial form or the true mould.

Another mould which behaves in a similar way, and produces even larger ferment cells, is the red mould of barley, Fusarium Hordei (see also Plate IV): like Mucor Racemosus it yields but a small percentage of alcohol. Brefeld found that most of the moulds were able to give rise to what one might term pseudo-Saccharomycetes, or cells having more or less resemblance to yeast in appearance and possessing in a varying degree the power of inciting alcoholic fermentation. The old term Torula applies conveniently to some of these growths which may very closely resemble Saccharomyces in shape and function and yet do not sporulate and are not regarded as true Saccharomycetes, although one must at least call some of them alcoholic ferments. One can only remark again that the natural linking up of different genera of living things stands as a kind of remonstrance to rigid classification.

TORULA.

Whilst pursuing researches on Saccharomyces one frequently encounters cells which rather closely resemble yeast, and to which Pasteur applied the name of Torula. They are to be found in maltings, on damp walls and floors, and in badly cleansed pipes in the brewery. These cells are widely disseminated and proceed from many varieties. They are generally of a spherical shape, but may be somewhat elliptic: the smaller varieties range in size from $1\frac{1}{2}$ to $4\frac{1}{2}\,\mu$, whilst the larger ones are from 3 to $8\,\mu$. The cells bud like yeast, but do not form ascospores under the ordinary conditions. The Torulae tend very often to form a film on the surface of a nutrient liquid and to creep up the walls of the containing vessel. As a rule they produce little or no alcohol, but there would appear to be one or two varieties of rarer occurrence which can ferment grape sugar and maltose, and give rise to a considerable percentage (6-7 per cent.) of alcohol. Cells of Torula seldom secrete the substance invertase—which is a well-known feature of Saccharomyces Cerevisiae—consequently they are unable to ferment cane sugar. A species of Torula is known that possesses a distinct reddish colour which is plainly visible when the cells are numerous in a flask, &c., but cannot be distinguished by the microscope in the single cell. This Torula frequently makes its appearance in flasks containing wort which has been infected by

contact with the air: in the majority of cases the cells multiply with some rapidity, but without giving any of the usual signs of fermentation and with the production of little or no alcohol. Torulae exhibit vacuoles, and sometimes these contain oil or fat globules of a slightly greenish or yellowish tint.

We have thus made an attempt to illustrate the chief variations in form that yeast may exhibit, by describing some of the better known species and varieties, and have moreover taken into consideration certain organisms which are able to function as alcoholic ferments although not true Saccharomycetes. Some of the more recently discovered Saccharomycetes furnish fresh forms, and we may regard it as certain that there will be further additions.

We have already said enough to indicate that the cells of different yeasts cannot be distinguished with certainty if one relies on the microscope alone as a means of identification; it therefore follows that not only must the shape of the cells be taken into account when investigating yeast, but also such other physical attributes as may be discoverable. There is something still to be said, however, about the shape of the cells. Every organism tends to reproduce itself in conformity with a particular type, and this conformity to type is the more likely to be maintained if the environment—that is to say, the conditions under which growth takes place—is uniform. Perfectly uniform in all the possible relationships one can hardly expect it to be, even for the lowest organisms, and consequently we have herein an element that must be reckoned with even with such a simple organism as yeast. In a general way one would be inclined to assume that the worker bees in a hive were practically alike, and that what one bee selected at hazard could do, any other of the community could effect just as well. We know, however, this is not the fact, but that instead some of the insects are more fitted by the operation of natural laws to fulfil their destiny than others, and that these individuals exist the longest if the environment becomes adverse. It will be discerned that we are practically stating the law of the 'survival of the fittest.' An organism so low in the scale of living things as the yeast cell might be expected to agree more closely with the typical form of the species it belongs to than an insect like the bee, the conditions attending growth being relatively simpler. Doubtless this is so, and the law of continuity is fully borne out; but yeast in common with other organisms is subject to another law, 'the law of variation,' which determines the fact that the natural characteristics are not

constant ; not only because the environment cannot be made absolutely uniform, but because there is a tendency in all living things to develop different characteristics from those possessed by their progenitor or progenitors, which finds expression, usually, in an accentuation of such qualities or attributes as are essential to the physical well-being of the organism. These laws have not only to do with the shape of yeast cells but with the functions; we may, however, for the time exclude the latter consideration and deal with the former. If we take a yeast such as S. Pastorianus we find that a free growth of it may furnish cells which depart somewhat widely from the typical form; not only so, but subsequent fermentations may favour the development of the form that is not typical or less typical. Again, there are varieties of S. Pastorianus which, although tending to exhibit a typical form under anything like normal or favouring conditions, may exhibit forms which merge into each other to such an extent, that it would be impossible to say from the microscopical appearance alone what variety of S. Pastorianus was present, or indeed whether S. Pastorianus were present at all. The conditions then which will affect the visible shape of yeast are (1) the particular species ; (2) the particular variety of the species ; (3) the conditions attending growth : and having dealt with (1) and (2) we may affirm that (3) may be regarded from two points of view, namely, first, the physical conditions, and, secondly, the chemical conditions. We will relegate the chemical conditions to a future chapter, and only speak here of any influence or action on the shape of yeast cells due to the natural forms of energy, electricity, light, and heat.

It may be considered that light has no determining influence on the shape of yeast cells. It has little or no effect, of any direct nature, on the functions of yeast and on fermentation as a whole, neither can one ascribe any effect in the same directions to the ordinary atmospheric manifestations of electricity. Heat, however, has a marked effect, and by applying a high temperature to yeast very considerable divergences in shape may be obtained. This is the case with beers submitted to the forcing process, the deposits of which very frequently exhibit cells which may be abnormally large or of very peculiar shape, and are not unfrequently an exaggeration of the typical form of the species.

The writer has obtained unusual forms by submitting a Burton yeast diffused in water to a temperature of 90° F. for one hour, afterwards setting the yeast in beer wort. Long pointed cells were

obtained, like S. Apiculatus on a very large scale. Excepting for some approach to these shapes in forced beers such cells are not found in Burton yeast or ales under any other conditions, and it is reasonable to conclude that the shape was modified by the preliminary heating. In forming conclusions about the morphology of a yeast the question of the temperature at which it has been produced should be taken into account. If the cells are not modified in shape by temperature they are frequently altered in other features, such as the appearance of the cell wall, the protoplasm, and more especially the vacuoles.

Experimental Work in connexion with Chapter III.

Secure if possible spontaneous fermentations in the juices of crushed fruits, e. g. grapes, strawberries, cherries, &c., as obtainable, separating the juice roughly from the other matters by pressing in muslin or linen. Put the juice so obtained into small flasks or bottles plugged somewhat tightly with cotton wool, and leave to such fermentation as may set in when the flasks are left in a warm, but not hot place. Examine the deposits at the close of fermentation.

Extracts may also be made by macerating figs, dates, or raisins in water and separating the sugary liquid as in the case of fresh fruit. Leave the liquids to ferment spontaneously.

Examine the sediments from the following liquids:—

Lager beer, if a bottle is obtainable showing sediment.

Deposits of ordinary bottled ales.

Sediments or deposits of cider, perry, and home-made wines in which fermentation is still progressing or not quite finished.

Yeast from breweries in different parts of the kingdom.

Mould growths sown in fermentable liquids, such as boiled raisin wort, sterilized grape juice, or solution of malt extract made with boiling water.

CHAPTER IV

THE EFFECT OF PHYSICAL AND CHEMICAL INFLUENCES ON THE YEAST ORGANISM

WE have now arrived at a stage where the general functions of the yeast cell may be taken into consideration, and a fuller degree of attention be paid to the physical conditions under which the organism lives and those that may attend or influence its growth.

In order to make the matters which follow clearer, it would be as well to take a rapid survey of the chief visible and readily ascertainable features exhibited by fermentation in bulk; that is to say, on the scale on which it would be carried out in a brewery or distillery. The things that may be perceived are these :—

1. Evolution of carbonic anhydride, with a corresponding appearance of 'head' or froth.

2. Formation of alcohol and ethereal products, with corresponding aroma. Rise of temperature, at first slow, then increasingly rapid.

3. Diminution of the specific gravity of the liquid as the percentage of alcohol increases.

4. Production of yeast, in many cases visible on the surface of the liquid.

5. Slackening of fermentation, diminution in the amount of carbonic anhydride evolved, gradual falling back in temperature, whilst in the case of brewer's fermentations the quantity of yeast on the surface increases.

6. Quiescence of the liquid, accompanied by a certain amount of clearing due to both rising and falling of suspended yeast and the deposition of other matters which help to make the 'lees,' 'dregs,' or 'grounds.'

It is of course the yeast organism that is the active agent throughout the progress of the changes just outlined, and its growth is subject to, or influenced by, certain prevailing conditions, which may be referred on the one hand to the region of physical changes, and on the other to those which are more purely chemical. Our present aim is, however, rather to deal with the physical side of the question,

although it must be remarked that the two sides are somewhat closely interwoven.

We have, as we now know, to do with a living organism whose visible mode of life is bound up with alcoholic fermentation. In various saccharine liquids it finds the different sorts of food it needs, and, speaking in general terms, there are three classes of nutrient matter—

1. Fermentable sugars.
2. Nitrogen in some assimilable form.
3. Mineral matter.

The fermentable sugars may be regarded as the heat producers, whilst the nitrogenous organic matters and the mineral matter go to build up the general structure of the cell, aided by the sugars, which, during their decomposition by yeast, supply the means of sustaining the temperature which is necessary for the proper exhibition of its vital force and energy.

The alcohol and carbonic acid formed may be regarded as excretory products of the organism.

The general similarity between the food requirements of the yeast cell and the needs of more highly organized living things, is rendered perhaps the more striking by the fact that a supply of air or oxygen is absolutely necessary for the prolonged healthful activity of yeast. The power that yeast possesses of absorbing oxygen can readily be proved in more than one way: for instance, the substance haemoglobin, derived from the blood, is capable of attaching oxygen to itself, and, when oxygenated, possesses the ruddy colour of arterial blood. Now if yeast be diffused in a solution of haemoglobin in this bright red condition, the tint rapidly changes from red to dark blue or black. If the yeast be removed by filtration, and the solution shaken up once more with the air, the original bright red colour is restored. The same result can be arrived at by enclosing the yeast in a porous membrane, such as bladder or intestine, and immersing it in the solution of haemoglobin, when it is found that absorption of the oxygen by the yeast goes on through the membrane—an action very similar to that which takes place in the organs and tissues of animals when the red oxygenated blood from the lungs traverses the capillaries and passes into the veins, bereft of its oxygen and possessing the colour of venous blood. If the cells of S. Cerevisiae be introduced into a liquid containing oxygen in solution, such as pure water saturated with the gas, the oxygen is absorbed with great rapidity, and sooner or

later an equivalent amount of carbonic anhydride is furnished by the cell. This constitutes a kind of breathing, and this respiration of oxygen is absolutely necessary for the continued well-being of the cell, for in absence of oxygen in some state, free or combined, but preferably free, fermentation languishes and comes to an end. Pasteur advanced the theory that fermentation was life without air, that is to say that the exclusion of oxygen was the predisposing cause of the yeast attacking sugar, the idea being that it was the oxygen contained in the sugar that the yeast was in search of, and that it went on chemically decomposing sugar to supply its continued need for oxygen. Interesting and suggestive as this hypothesis was, it can no longer be considered tenable in face of the results of more recent investigations. For vigorous fermentation a continued supply of oxygen is needed, and with a regulated amount, other conditions being suitable, vegetation or increase of the yeast and fermentation proceed together. If the supply of oxygen be limited, the amount of sugar fermented in a given time may be, for a while, increased; and if the supply of oxygen be made very copious fermentation is undoubtedly retarded; but in the latter case the ultimate result as regards the amount of sugar fermented will exceed that of the fermentation where the amount of oxygen is restricted. Adolf Mayer says that fermentation may be regarded as a kind of substitute for the respiration of oxygen, inasmuch as it is carried out more actively by individual cells of strong vitality the more fully the respiration is suppressed, and in so far as the individual cells possess the capability of carrying on fermentation for an extended period following the exclusion of air or oxygen: excepting that the substitution of fermentation for respiration is not entirely adequate for all the functions of the cell. We have thus a conception of yeast as an organism which, in a manner, breathes oxygen or air, the oxygen so utilized being absolutely necessary to it. Schützenberger concluded that when fermentation was at the point of the highest activity, the maximum of the respiratory action is also reached; and it may be adduced in connexion with this, that it is a common experience in certain yeast manufactories where fermentations of an exceedingly vigorous kind are secured, that in order to produce the best results as regards quantity and quality of the yeast, it is necessary to pass a current of air through the fermenting liquid. We will leave this important function at this point, and direct our attention to other attributes of the yeast organism and to points connected with the maintenance of vitality.

The production of heat during fermentation is a very interesting phenomenon, and is the usual result in breweries and distilleries where saccharine liquids (worts) are fermented in bulk. As the temperature rises the fermentation visibly becomes more and more energetic, the fermentable constituents of the wort disappearing at a corresponding rate. At first view this production of heat is a rather curious fact, or rather, it is strange that yeast should require to break up so much sugar as to chemically liberate this heat; for the decomposition of sugar is an *exothermic* reaction; yet if one considers yeast as a *living* organism, there is a certain naturalness about the phenomenon. The honey-bee will again furnish us with a good illustration of the difference between the traceable vital phenomena of the one individual and the many. It would not, for instance, be an easy matter to ascertain that a bee had a normal temperature comparable with that of human beings, and a very special clinical thermometer would have to be designed to discover the fact, but the temperature inside a hive of bees shows plainly what the collective effect of the units is. The same thing may be said of yeast, for it is a fair argument that the increasing temperature of a bulk of liquid in a state of fermentation is due to the total effect of the millions and millions of cells present, each of which has a *normal* temperature at which its vital processes are most active, and this temperature may be taken as approximating to 86° F. (Märcker and also Pedersen; Lintner gives 96° F.). It is conceivable therefore that the yeast cell may have an internal temperature differing from the temperature of the liquid in which it is displaying its energy, and that in the majority of cases—especially in brewer's fermentations—it is parting with heat, resulting from the decomposition of alimentary substances, to warm up the liquid to its own normal, and as the liquid becomes warmer the organism develops its energy more and more in the direction of promoting those changes in the liquid which are summed up in the word 'fermentation.' Putting the case in another way:—Whilst the organism is continually parting with heat it is numbed and sluggish, but as it begins to be able to store some of the developed heat its vitality actively increases and goes on increasing up to the point of its maximum activity, which is about 86° F.[1] Fermentations may spontaneously reach a higher temperature than this

[1] The thermo-chemical side of the liberation of heat during alcoholic fermentation has been most admirably dealt with of late by Prof. Adrian J. Brown (vide 'The Heat of Fermentation,' *Trans. Fed. Insts. of Brewing*, vol. vii. No. 1).

(a temperature of 92° F. is recorded in some distilleries), but it does not follow that the highest temperature attained represents the *normal* of the yeast; for it should be remembered that the cell is not only surrounded by the materials it is feeding upon, but also—in a large measure—by the excretory products of its own vital processes, and it is quite conceivable that the prejudicial effect of these substances may determine, as it were, a fever temperature of the yeast cell and a state of things in which its vitality is soon very greatly impaired. The yeast may in fact be partially poisoned by the excretory products, not only by direct contact with them, but also owing to the fact that their presence would render it very difficult for the cell to gain so much nutrient matter from the fermenting liquid as it could were they absent.

The next point for consideration is:—How does the yeast deal with the matters in a nutrient and fermentable solution? Since the cell envelope is continuous, and there is no aperture corresponding to a mouth, it follows almost of necessity that either the cell can by some inherent power deal with the substances it modifies outside itself, or they must be taken into the cell and dealt with there. In either case a certain permeability of the cell membrane is essential to the vital processes; for it is hardly conceivable that the organism can build up its tissue and elaborate the various components of the protoplasmic matter, without a passing of the matters it requires inwards, and of its excretory products outwards; at the same time it is not impossible. Liebig's first conception of fermentation was, 'that the molecular structure of bodies capable of fermentation was easily upset, and consequently a mechanical impulse sufficed to cause a rearrangement of the atoms, this being done by the exciter of fermentation, the ferment. The ferments are not stable substances, but all nitrogenous organic substances in a state of decomposition are in a position to transfer their decomposition to fermentable substances.' Now, after Pasteur had shown that yeast was a living organism, Liebig modified his conception of fermentation in the following way. He considered that fermentation and the renewal of the organism stood in no direct relationship, and were two distinct processes; further, that yeast consisted of vegetable cells which increased in a liquid when sugar, albuminoids, and mineral matters were present in it. The chief part of the cell contents consists of a substance similar to albumin combined with sugar. As soon as the yeast has completed its growth and rests, a molecular action is set up in the cell contents, giving rise

to alcohol and carbonic anhydride from the combination of albumin and sugar just mentioned. Sugar drawn into or diffusing into this centre of activity becomes split up into alcohol and carbonic anhydride. Fermentation is thus a resultant of the dying of the yeast. Summarizing the foregoing, growth and organization of yeast on the one side, and the exciting of fermentation on the other, are two processes which should be widely separated from each other; the latter process begins with the cessation of the first. This was Liebig's later theory of fermentation, and we shall see that the latter part of it—the summary, in fact—is no longer tenable in view of more recent knowledge. We need not discuss it, but may pass on to Nägeli's researches and his well-known vibratory theory, which may be briefly expressed thus:—Fermentation is caused by certain molecular vibrations of the components of the protoplasm of the cell which are communicated to substances outside the cell: the vibratory impulse so communicated disturbs the equilibrium of neighbouring substances and causes decomposition of the latter, giving rise to the phenomenon of fermentation. This theory of Nägeli bears an undoubted resemblance to one part of Liebig's hypothesis, that is, in so far as the mechanical impulse is concerned; but Nägeli evidently looks to the outside of the cell for the manufacture of the products of fermentation, whilst Liebig regards the inside as the workshop. Before proceeding further let us endeavour to set the idea of mechanical impulse in a clearer light:—The changes in molecular constitution set in train by communicated impulse or vibration may not altogether inaptly be illustrated by the familiar scientific toy known as the kaleidoscope. A certain number of pieces of plain and coloured glass, wire, &c. (which may be regarded as representing chemical atoms) are, by the mode of construction of the instrument, caused to display symmetrical forms (chemical molecules, or groups of atoms arranged symmetrically). The vibratory impulse is represented by the turn or shake given to the tube, which induces a change in the pattern. The change may be of two kinds: (1) the rearrangement of the whole of the pieces (atoms) in a new symmetrical form (molecule); or (2) to build up a symmetrical pattern with part only of the original pieces, the pieces not in view having an equal capacity for giving a symmetrical pattern if they were in view. We thus have an illustration of the way in which atoms may be rearranged, forming a fresh molecule or, on the other hand, a breaking up of the original molecule into other molecules. It should be observed, however, that the kaleidoscope pattern is only

an apparent pattern in one plane, whilst the molecule of a substance like sugar may have its atoms disposed in several planes.

Now Nägeli finally came to the conclusion that fermentation takes place outside the cell, and that the action extends to a distance of one-fortieth to one-fiftieth of a millimetre from the cell wall. The experimental evidence on which his conclusion is based is afforded by the behaviour of certain fruits when immersed in grape juice: the flesh of the fruit shows the production of a considerable amount of alcohol before any trace of fermentation is visible in the juice itself. If, however, the fruit is skinned before immersion in the liquid, no formation of alcohol in the flesh of the fruit takes place. Nägeli distinguishes this from the self-fermentation of living cells which has been alluded to in an earlier chapter, and regards the fermentation in the first case as due to the vibrations of the yeast cells existing on the skin of the grape, being transmitted through the skin to the fermentable matter inside. He sums up the question thus:—The causes of fermentation are found in the living plasma in the interior of the cell, but the action extends for a certain distance (at least one-fiftieth of a millimetre) outside the cell. The decomposition of sugar takes place to a small extent in the interior of the yeast cell, but chiefly exterior to the cell. This holds good for both the chief products of alcoholic fermentation, alcohol and carbonic anhydride: the by-products, glycerine and succinic acid, are probably formed in the interior of the cell.

This ingenious and complex theory of Nägeli has comparatively few adherents: the general consensus of opinion is in favour of the changes, which yeast inaugurates and maintains, being conducted inside the cell; and there can be little doubt that *diffusion* plays an important part in the life-processes of yeast. To this particular matter we will therefore direct our attention. Diffusion is a property possessed by tissues and membranes by virtue of which they allow certain substances in solution to pass through them; not, however, in the sense of water running through a fine sieve, but rather in the passing on of moisture from cell to cell in any given tissue or membrane, which results in the whole becoming moist. A large class of substances which, speaking generally, are such as can be obtained in a crystalline condition (crystalloids) are susceptible when dissolved in water of being carried through the permeable membrane or tissue with the water, a process which is also termed 'osmosis.' Another class of substances, generally non-crystallizable bodies (colloids), cannot pass in this way: they are non-diffusible, or not subject to osmosis. A solution of cane sugar

affords a good example of a readily diffusible substance, whilst a solution of gum arabic provides an illustration of the opposite characteristic or colloidal state. In addition to this action certain substances, e.g. strong saline solutions enclosed in membranes the other surface of which is in contact with water, have a strong tendency to draw water in at a greater rate than saline particles can pass out, thus giving rise to a state of internal pressure. This state of pressure or turgescence is a normal condition in the cells of living plants, and indeed seems to be a necessary adjunct of energetic vitality. Applying these principles to the yeast cell it is apparent that diffusible substances—and the sugars and mineral compounds are included in this category—could pass into the cell (endosmosis), and that alcohol and carbonic anhydride, also diffusible, could pass out (exosmosis), whilst other substances would, in consequence of their non-diffusibility, remain outside the cell unless changed or modified into diffusible substances; and the non-diffusible portion of the protoplasm of the cell would remain inside the cell. This view of the matter is plainly separable from the idea of molecular changes, set up by vibrations inside the cell, being communicated to substances external to the cell. Both actions are possible, and, in so far as *internal* vibrations of the protoplasm are concerned, even probable. In the present state of knowledge it rather simplifies matters to adhere to the osmotic explanation of the way in which the yeast cell receives the matters it needs, and parts with those which are not necessary or excretory. There is every probability that fermentation takes place in the interior of the yeast cell. A truly remarkable discovery, which has a very distinct bearing on this question, has been made in recent years by Edward Buchner. By triturating yeast with sand and mixing it with *kieselguhr* (diatomaceous earth), and then submitting the mass to enormous pressure, Buchner succeeded in expressing from the yeast a viscid yellowish liquid which possesses the remarkable power of inducing alcoholic fermentation in solutions of the ordinary fermentable sugars, carbonic anhydride being evolved. Buchner believes that the active agent in the liquid is a nitrogenous compound of the nature of the enzymes. He terms it zymase. This substance is no doubt being continually formed in healthy, well-nourished yeast-cells, and doubtless attacks the sugar which has diffused into the cell. It should be remarked that Buchner's discovery verifies in a manner the speculations of some of the scientific men who have given great attention to the vital processes of the yeast cell. Liebig's later hypothesis virtually includes this idea. Traube and Hoppe-Seyler

also held it, for they say 'in the yeast cell, amongst many other substances, there is one present which acts as a ferment on sugar and splits it up into many compounds.' Put into other words this implies that the Saccharomycetes secrete a definite alcoholic ferment, and Buchner has proved that this is indeed the case. Most interesting consequences are following and will follow this remarkable discovery: one should not forget, however, that though it pushes the cause of alcoholic fermentation a little further back there is no reason to believe that Buchner's zymase could be produced by any other than vital agencies or in association with living matter.

Some attention may now be bestowed on the ways in which yeast may be influenced by changes in the physical conditions to which it is subjected.

First, as regards temperature, we have seen that yeast is capable of displaying a maximum of fermentative vigour at temperatures that are relatively high, and this probably holds good for most species and varieties of yeast. At the same time the range of temperature, throughout which yeast is capable of displaying the phenomenon of alcoholic fermentation, is a very wide one, for it begins at or about the freezing-point and can, as we have seen, go up to 100° F.: above this temperature the vitality of the yeast is rapidly impaired, and very few species of yeast resist an exposure to a temperature of 120° F. for more than about one hour: five minutes' exposure to 140° F. will kill practically all yeasts. This is in the moist state; in a desiccated condition they can sustain a short exposure to a temperature of 212° F. (100° C.) without being killed, and in either the dry or moist condition can withstand a great degree of cold—as low as – 130° C.

As regards the influence of temperature on the activity of fermentation it is sufficient for present purposes to say that the fermentative power (indicated by the amount of sugar fermented by a given weight of yeast in a given time) rises with the temperature: not however in direct proportion, but at a rapidly increasing rate, as the temperature of the fermenting liquid approaches the optimum temperature of the yeast (86° F.). The rise of temperature has also a very marked effect on the rate of increase of the sown yeast, as the following results of Rasmus Pedersen will plainly show:—

One hundred cells sown became in the same period of time (and presumably under similar conditions)

225 cells at 4·0° C.
476 ,, 13·5° C.

1206 cells at 23·0° C.
1759 „ 28·0° C. (83° F.)
639 „ 34·0° C. (93° F.)
100 „ 38·0° C. (100·4° F.)

And again:

Time required for the completion of a generation of yeast cells :—

20 hours at 4·0° C.
10·5 „ 13·5° C.
6·5 „ 23·0° C.
5·8 „ 28·0° C. (83° F.)
9·8 „ 34·0° C. (93° F.)

Regarded from the point of view of such results it is plain that lager-beer fermentations, which are carried out at low temperatures (about 40° F.) and last for many days, are but sluggish fermentations when compared with those by which English beers are produced, the average temperature for which is about 65° F., and the time four or five days.

The Action of Light.

Light has little or no effect on the vitality of yeast and the progress of fermentation, and in this connexion it may be said that as a vegetable organism, yeast displays the characteristics of a fungus. Being devoid of chlorophyll there seems no particular reason why it should not grow just as well in darkness as in light; at the same time it is a question whether yeast could go on indefinitely propagating itself in darkness without deteriorating. This consideration must, of course, be taken exclusive of any indirect influence that light is able to exercise, such as the suppression of bacteria, or chemical changes induced in the liquids undergoing fermentation. Martinand says that if yeasts are maintained at a temperature of 40° to 45° C. (104° to 113° F.) an exposure of four hours to direct sunlight will kill the cells, and that three days at a temperature of 36° C. (97° F.) will give a like result; without sunlight the cells will survive at the latter temperature for a long period.

The Influence of Electricity.

Many of the old works and treatises on brewing contain references to the supposed action of atmospheric electricity upon fermentation, as also upon beer and other fluids. In a work on brewing, published

as recently as 1875, a good many pages are devoted to a consideration of all kinds of suggested possibilities and probabilities in connexion with thunderstorms—currents of electricity through the ground, association of different metals in the fittings of fermenting vessels, and so on—giving rise to the reflection how fast and far one may ride on a theory, especially a wrong one. Electricity has been held responsible for a variety of effects on fermentation, such as unhealthy appearance of the frothy head, sudden stopping of fermentation or unwonted acceleration, cloudiness, haziness, and last, but not least, souring.

In 1884 Dr. Squire[1] described some experiments which he had made with the object of discovering if electric currents passed through fermenting liquids produced any effect. The results he obtained showed that no appreciable effect followed either in respect to the yeast cells or as affecting other organisms, e.g. bacteria, which were invariably associated with the yeast. It is true that other investigators, Cohn and Mendelssohn, showed that a liquid containing bacteria can be sterilized by a powerful current of electricity (when a weaker current was employed there was no effect): but Dr. Squire ascribes this fact to the septic or bactericidal properties of some substance produced by the electrolysis, e.g. hydrogen peroxide, and on repeating the experiment with a liquid containing both yeast and bacteria he could not sterilize it owing, as he supposed, to the decomposition of the bactericidal hydrogen peroxide by the yeast, for it will be remembered that yeast has a powerful affinity for oxygen and can probably remove oxygen from the hydrogen peroxide (H_2O_2), leaving water (H_2O) in its place. Some of Dr. Squire's experiments have been repeated, and a good many fresh ones recently performed, by Mr. Alfred C. Chapman; the results are incorporated in a very interesting communication entitled 'Beer and Electricity[2].' Mr. Chapman summarizes his conclusions as follows:—

1. That electricity *quâ* electricity has no effect on the stability of beer (and consequently on other fermented liquids).

2. That the products of electrolysis (assuming the electric current to have been capable of producing an appreciable quantity of such) would exert but little influence. In any case this influence would be in the direction of retarding the changes due to bacterial activity.

3. That the quantity of ozone present in the atmosphere during

[1] *Journ. Soc. Chem. Ind.*, 1884, pp. 544, 545.
[2] *Journ. Fed. Insts. Brewing*, 1899, vol. v. pp. 278–92.

thunderstorms would be insufficient to produce any marked change. Any effect produced, as in the case of the products of electrolysis, would tend in the direction of increased stability.

4. That beer, like many other articles of food (meat, milk, &c.), is more likely to undergo change during warm thundery weather than at other time, is, of course, well known; but in none of these cases is electricity to be considered as either the direct or indirect cause. The explanation is, rather, that the atmospheric conditions which result in thunderstorms favour bacterial infection and bacterial growth. At such times a high temperature prevails and the air is laden with bacterial germs. The fall of the barometer, moreover, tends to bring out bacterially infected air from the upper layers of the soil, and distribute the germs it carries.

In face of these researches the long-cherished idea of electrical influence on beer is swept away, and the lucubrations of the older textbooks on brewing come to nothing. As Mr. Chapman suggests, the brewer is bereft of the time-honoured excuse that 'the thunder has turned the beer sour.' The well-informed and scientifically educated brewer can still solace himself and soothe his customers, however, by saying that 'it is due to the unfavourable atmospheric conditions which have prevailed of late'; that is, assuming that he knows of no other and more concrete explanation of the unwelcome fact that the beer is not all it should be.

Influence of Pressure.

The cells of healthy yeast are capable of sustaining enormous pressure without losing their vitality, if distributed fairly equally around the cell. Regnard has submitted yeast to a pressure of 1,000 atmospheres for an hour without impairing its vitality, and Melsens has applied 8,000 atmospheres without killing the cells.

Pressure exerted on a fermenting liquid does not materially retard fermentation until the pressure becomes high; for if a volume of fermentable liquid (beer wort, cane-sugar solution, &c.) be set with yeast and fermented in closed vessels connected with a pressure gauge [1], very high pressures are indicated. The writer has frequently obtained indicated pressures of 160 or 180 lb. per square inch, and the pressure seemed inclined to rise indefinitely; but it was deemed expedient to let the fermenting liquid escape at the maximum pressure

[1] *Brewer's Guardian*, 1887, p. 129.

registered by the gauge (180 lb. per square inch), although the vessel in which the experiments were conducted had been tested up to 250 lb. per square inch. Sudden release from pressure, resulting from the letting off of the liquid, bursts some of the weaker yeast cells. The general appearance of the yeast under the microscope was unsatisfactory; for some of the cells were abnormally large and very highly vacuoled, and cells were also visible in which budding had commenced, but the bud had stopped in its development and had become granulated. This development of pressure by fermentation was made the basis of a method for estimating the fermentative power of yeast, and both the apparatus and the method are fully explained in the Appendix.

In the same year (1887) as the experiments just alluded to were made, Prantl found that fermentation of a saccharine liquid is retarded when the process takes place in a closed vessel under a pressure of about twelve atmospheres; and he attributes the retardation to the fact that the pressure prevents the escape of bubbles of carbonic anhydride, and so the yeast cells settle at the bottom of the vessel, where they become inactive. Some further experiments on this subject were carried out in the laboratory of the Berlin Brewing Institute. Three equal volumes of the same brewer's wort were fermented under conditions as nearly alike as possible, except that one quantity was exposed to the air, another was under a constant pressure of carbonic anhydride, and the third under a partial vacuum produced by an air-pump. The last-named fermented the most actively, while that exposed to the air came next, and the fermentation under pressure was slowest. It was found, moreover, by direct experiment that agitation of the fermenting liquid does not compensate for the retarding influence of the pressure of carbonic anhydride. So that although Prantl's observation was quite correct as to the retarding effect, his inference as to its being due to the enforced quiescence of the cells was incorrect. The retarding is almost unquestionably due to the inhibitive effect of the gas, and it is readily conceivable that the free escape of carbonic anhydride, secured by a partial vacuum, should prove a very favouring condition for fermentation.

State of Moisture or Dryness.

Yeast in the thick creamy state in which it is usually obtained in the brewery contains about 14 per cent. of dry yeast. When in the pressed condition and apparently fairly dry it contains about 28 per

cent. of dry matter and 72 per cent. of water. In concentrated saline or saccharine solutions the percentage of water may fall to 25 per cent., but not below this. If other means be taken to remove water from the yeast and the percentage is brought down to 13 per cent., the yeast dies[1]. Yeast may be brought to somewhere near this point of desiccation, however, by careful treatment, and preserved for some years. Pasteur used plaster of Paris and incorporated the yeast with it, but other substances do just as well or even better, e.g. potato starch or maize flour. The yeast in a fairly dry pressed state can be kneaded up with these by hand until a dry mass is obtained, which should then be broken up as small as possible and dried in a current of air at 80° or 90° F., then carefully corked and sealed in a dry bottle. In this desiccated condition it can be exposed to all ordinary variations of temperature without the slightest risk of being injured thereby. Yeast may also be kept in sterilized dry filter-paper, and has been known to retain its vitality in this way for three years. All yeasts stored in these different ways take some time to come into a state of fermentative activity again.

Influence of Sound on Yeast.

Dumas found that sonorous vibrations have no influence on the movements of fermentation, and no experimental evidence has been since adduced to support the idea that fermentation is influenced by sound.

Influence of Agitation.

There is a preponderating weight of evidence to the effect that agitation is favourable to fermentation. Mr. L. Briant's experiments establish this, and Mr. J. O'Sullivan says that 'agitation accelerates fermentation whether it is carried on with or without excess of yeast.' A distinction must of course be made between a mere mechanical stirring or rousing of a liquid undergoing fermentation and the same thing done in such a way as to oxygenate or aerate the fermenting liquid, for in the latter case a fresh factor would have to be reckoned with. Any influence that keeps the yeast in suspension in a fermenting liquid must favour the accessibility of the cell to its nutrients. In the case of the coagulated yeasts, which tend to remain in a thick layer at the bottom of the fermenting liquid, the influence of mechanical stirring is especially favourable.

[1] Kayser, *Les Levures*, p. 150.

The Fermentative Power of Yeast.

A certain amount of controversial matter has been published regarding the question as to a correct definition of the fermentative power of yeast. The difficulty really commenced when Pasteur, in describing various experiments in which a certain amount of sugar was fermented by a given weight of yeast, deemed it unnecessary to take into consideration the time that had been consumed in the operation. Quoting Adrian J. Brown[1], 'there is little doubt that Pasteur viewed fermentative power as the total power exercised by a growth of yeast throughout its life-history in some fermentable and nourishing liquid; in fact, as a sort of increasing many-celled organism using sugar in various ways, according to its age or surroundings.' Adrian Brown goes on to say, 'Throughout my experiments I have considered fermentation as a product of the continued life of a non-increasing individual cell.' An attempt has been made to make use of both views by distinguishing between the terms in use as follows:—

Fermentative activity or fermentative value of a yeast is obtained by dividing the weight of sugar fermented by the weight of yeast present in the solution, as apart from the fermentative power, which means the weight of sugar fermented by a given weight of yeast in a given time. This distinction seems at best but a clumsy expedient and eminently confusing. It is certain, as Adrian Brown affirms, that the time factor cannot be ignored. Now it is easy to say that the fermentative power of a yeast is exhibited by the amount of sugar fermented in a given time by a given weight of yeast, but as a matter of fact it is by no means easy to ascertain the amount of work done by a certain number of cells, or per individual cell, unless the conditions are made such that the cells do not increase: for if they do increase one has obviously to deal with a different number of cells at all points of the fermentation up to the time the increase has stopped, which may be only with the close of fermentation. The condition of non-multiplying cells can be secured, however, and in this connexion we may again quote the same authority[2]:—'When any fermentable nutritive solution such as malt wort is inoculated with a "high" fermentation yeast, and kept at a temperature favourable to yeast growth, the cells reproduce themselves rapidl for a time and

[1] 'Influence of Oxygen and Concentration on Alcoholic Fermentation,' *Journ. Chem. Soc. Trans.*, 1892, p. 377.

[2] Ibid., p. 370.

then their reproduction ceases (this is apart from any question of "secondary" fermentation), and the fermentation of the solution may still be carried on by the continued life of the cells already formed.' One interesting point in connexion with the increase of yeast cells in a fermentable liquid is, that, although different liquids may cause yeast to increase to a differing extent, yet the same liquid, providing all the conditions, such as agitation, &c., are kept constant, appears to cause an increase in the number of cells to about the same maximum, no matter how the amount of yeast added to start the fermentation may vary (it is assumed that the same yeast at the same age is used). This maximum number of cells equals about twenty-five cells per standard volume[1] ($\frac{1}{4000}$ of a cubic millimetre). If, therefore, a given volume of fermentable liquid be seeded with yeast cells in this relationship, the condition of non-multiplying cells is secured and the amount of sugar fermented in a given time can be referred to a known and constant quantity of yeast. Whilst prosecuting these and allied experiments Adrian Brown found that oxygen favoured fermentation by increasing the fermentative power of the yeast. This conclusion has been denied by some, but supported by a majority of investigators, and so, as we have already said, Pasteur's view, that fermentation was life without oxygen, is no longer tenable: the fact is that yeast cells can use oxygen in the manner of ordinary aerobic fungi and require it for the full completion of their life-history, but the exhibition of their fermentative functions is not dependent on their environment with regard to free oxygen. Adrian Brown's conclusions were more firmly established by a further research[2] entitled, 'The Specific Character of the Fermentative Functions of Yeast Cells,' and his fresh experiments showed that there is no direct proportionality between the weight of yeast formed and of sugar fermented. He apparently prefers the expression *fermentative activity* to express the fermentative power of an organism in a unit of time.

[1] Twenty-five cells per $\frac{1}{4000}$ c.m. = 100,000 per c.m.
= 100,000,000 (one hundred millions) per 1 c.c.,
or 10,000 millions per 100 c.c.;
and this × the weight of 1 cell, ·0000000005 gram = 5 grams per 100 c.c. So that 5 grams per 100 c.c. is the limit of production of yeast for any liquid. This is equal to about 7¼ oz. on an average per gallon, or 16¼ lb. average per barrel, as pressed yeast.

[2] *Journ. Chem. Soc. Trans.*, 1894, p. 911.

CHAPTER V

CHEMICAL SCIENCE

WE have now reached a point when, in order to make clear what follows, it is desirable to obtain a general idea of the scope of chemical science. We will therefore devote this chapter to such chemical considerations as will serve to elucidate many of the phenomena of fermentation, and the changes inaugurated by fermentative processes.

Chemistry deals with the composition of all kinds of matter, and takes cognizance of the changes which matter undergoes in composition, due to the influence of the various forms of energy, light, heat, electricity, &c., and the chemical interaction of substances.

Taking a general survey of the known forms of matter we find an enormous range of substances, having simple forms at one end of the scale and compounds of the utmost complexity at the other. Some seventy or more substances are known, including metals and non-metals, which cannot be resolved into anything simpler by any known means. These are called *elements*, and as examples we may adduce such familiar substances as lead, copper, iron, zinc, aluminium, magnesium, and the less common metals, sodium and potassium; whilst among the non-metals, hydrogen, oxygen, nitrogen, carbon, sulphur, and phosphorus may be given. Any one of these substances may be submitted to all known chemical and physical processes without being resolvable into two or more components: changes, however, in the direction of building up these elementary forms into more complex ones by chemical union are easily secured. We may here at once draw a distinction between a mere mechanical mixture of substances and substances in a state of chemical combination. A good illustration is furnished by sulphur and iron in a fine state of division. If the two substances be mixed a greyish powder results, from which the iron can be abstracted by a magnet or the sulphur obtained by a solvent such as carbon disulphide. If heat be applied a new

substance is formed, viz. sulphide of iron, having new properties, and from which the sulphur and the iron can no longer be obtained by the processes which sufficed to separate them prior to heating. The iron and sulphur have entered into chemical combination, and the two elements have formed a compound. Concerning the nature of the particles of each and every substance, chemical science rests on the well-founded hypothesis that there are two forms of division, named respectively *atoms* and *molecules*. An atom is scientifically defined as the smallest amount of matter which can enter or leave a chemical compound, i.e. can enter into chemical change, chemical affinity being the force which holds the diverse atoms in a compound together[1]. The chemical affinity of the elementary substances towards each other varies very greatly indeed: where it is strong it means that the substance formed by the union is only decomposable with difficulty, and, conversely, where the affinity is weaker decomposition is easier to effect.

The atoms of every substance must be regarded as possessing some kind of polarity, or a power of attraction for other atoms, the tendency being, doubtless, to attract an opposite polarity just as the north pole of one magnet attracts the south pole of another. The result of this is that all liberated atoms tend to group themselves in systems or small masses which are called molecules. Where the atom displays a combining power equivalent to that possessed by one atom of hydrogen [examples: hydrochloric acid (H – Cl); water (H – O – H)] it may be considered that it has, as it were, a single pole and requires an opposite polarity, either existing or induced, to establish equilibrium. This is exemplified by the hydrogen molecule, which contains two atoms of the element which may be graphically expressed thus:

The hydrogen molecule is a definite mass of matter whose weight can be definitely determined, and all substances are collections of molecules, and in these molecules the qualities of the substance inhere: so that a molecule may be defined as the smallest quantity of

[1] The chemical atom is something inconceivably small and goes far beyond anything suggested by the mechanical division of a substance, even as carried out under the highest power of a microscope. A particle of matter just visible when magnified, say 3000 times (diameters), might contain a huge number of chemical atoms.

a substance which can exhibit the chemical and physical characteristics of the substance. Molecules, however, are destructible, whilst atoms are indestructible, and chemical change consists in the production of new molecules by the rearrangement of the atoms of former ones. The illustration we employed in a former chapter, of the kaleidoscope, comes in not inaptly here also.

A starting-point in the exposition of the molecular theory is afforded by the great generalization known as Avogadro's law (1811), which for half a century received but little recognition. It is as follows:— Equal volumes of all substances in a state of gas or vapour contain, under similar conditions, an equal number of molecules. Now if some standard of weight can be constituted in connexion with a known gaseous substance, the weights of all gases and vapours can be referred to it, and so the weight of the molecules obtained. The selected standard is hydrogen gas, one litre of which under standard conditions weighs ·0896 gram. A litre of oxygen gas under the same conditions weighs 1·4336 gram ($=·0896 \times 16$); and if the weight of hydrogen be taken as unity, then the relative weight of oxygen $=16$; and since the molecules of both hydrogen and oxygen contain two atoms, the molecular weight of oxygen becomes $16 \times 2 = 32$, and of hydrogen $1 \times 2 = 2$. The number 16 represents then the atomic weight of oxygen referred to the hydrogen atom as unity.

Although molecules are infinitesimally small, they are not immeasurable, and to the chemist and physicist they are real magnitudes, which, as Dr. Cooke says, 'are no further removed from our ordinary experience on the one side than are the magnitudes of astronomy on the other.' In regard to their properties and relationships we have definite information. At present we have only taken the hydrogen molecule at the arbitrary value of unity, and the question arises as to whether any idea of the absolute weight or size of a single molecule of hydrogen or any other substance can be gained. The calculation of Sir William Thomson (now Lord Kelvin), based on the theorem of Clausius, gives the number of molecules in a cubic inch of a gas as 10^{25}, or about one hundred thousand million million million (at 0° C. and 760 mm. pressure). It is plain, then, that there is but little chance of the hydrogen molecule ever being rendered visible to human gaze, neither is the balance known that could weigh a single molecule, or even a round million of them.

The molecules of a substance are considered to be in a state of constant motion, the rate of motion varying according to physical

state and temperature ; the motion ceases only at the temperature of absolute zero (– 273° C.). In a gas, the movements of the molecules are supposed to take place in straight lines, the molecules rushing to and fro between the walls of the containing vessel. The mean velocity of the molecules determines what is called the temperature of the substance, and the higher the temperature the greater is the mean velocity. The mean velocity of the hydrogen molecule at 32° F. and 30 in. barometer = 6097 feet per second.

Since all substances are not obtainable in a state of gas or vapour, the weight of the molecules as compared with the hydrogen molecule is not obtainable in the same way, and so chemical methods have to be resorted to which are adequate to give the molecular weight of the particular substance.

We have spoken of the hydrogen molecule as consisting of two atoms, so that the hydrogen atom must be regarded as the half-molecule, and this consideration holds for some other substances, e.g. oxygen, nitrogen, and chlorine gases, whose molecules may be thus expressed (the disk with the symbol inside representing the single

atom of the particular element). It will be remembered that we have defined the atom as being the smallest quantity of matter that can enter into chemical union. This may be exemplified by the following compounds: hydrochloric acid (H – Cl), in which a single atom of hydrogen is united to a single atom of chlorine ; water (H – O – H), in which one atom of oxygen combines with two atoms of hydrogen ; ammonia gas (H – N – H), in which one atom of nitrogen is combined

H

with three atoms of hydrogen ; and carbonic anhydride (O = C = O), in which one atom of carbon combines with two atoms of oxygen. All the elementary substances have symbols assigned to them, and they not only stand for the names of the elements but express one atom, and the weight of the atom is called 'the atomic weight.' For reasons just put forth the atomic weight of an elementary substance is generally half the molecular weight. The elements also have different combining powers, the unit of combination being the combining value of hydrogen, which is monovalent or a monad. In the examples just given it will be seen that oxygen has the power of attaching two monad atoms to itself; it is therefore called bivalent or a dyad.

Nitrogen in the compound given is a triad (it can also figure as a pentad); and carbon is a tetrad, the two oxygen atoms in the compound CO_2 satisfying four powers of attachment or 'bonds,' as they are often called. The use of the symbols enables one to write down all traceable chemical reactions in a short, convenient, and scientifically accurate manner.

Changes induced by chemical processes are either in the direction of simplification or of the opposite character, viz. the building up of more complex substances than those which form the starting-point. The first order of changes is defined as analytical, and the second synthetical. The elements of every chemical change are (1) one or more substances, called the factors, with which the change begins; and (2) one or more substances, called the products, with which the change ends. For example:—

Calcium Sulphate	Sodium Carbonate	gives	Sodium Sulphate	Calcium Carbonate
$CaSO_4$	+ Na_2CO_3	=	Na_2SO_4	+ $CaCO_3$.

Chemical processes are frequently accompanied by striking physical phenomena, such as the development of electricity, heat, and light, but these belong to the region of physics and are susceptible of examination by the physicist. What the chemist takes cognizance of is the change of substance. Some chemical processes are easy to follow, but this is by no means the rule, especially when the oxygen or the carbonic acid of the air take part in a chemical change, or where the products of a chemical reaction are wholly or partly gaseous (both these considerations apply in fermentative changes).

A substance too may be decomposed under very different conditions: if wood or coal be burnt in free air the only visible substances produced are possibly a certain amount of smoke and some ash; but if heat were applied to the wood or coal in a closed vessel, say the retort of a gas-works, a quantity of tar and charcoal or coke could be obtained, and the tar would, if chemically dealt with, furnish a host of substances.

In every chemical reaction the sum of the weights of the products is exactly equal to the sum of the weights of the factors. This fundamental chemical truth is known as the law of conservation of mass, and is susceptible of comparatively easy chemical proof. It is also a truth, and an exceedingly important one, that in all chemical processes the weights of the several products and factors stand in a definite relationship to each other. In the experiment given

a little further back, where the mixture of finely divided iron and sulphur was converted into a compound by heat, the iron and the sulphur enter into chemical union in certain definite proportions, and these proportions are one atom of iron (weighing fifty-six times as much as one atom of hydrogen) and one atom of sulphur (weighing thirty-two times as much as one atom of hydrogen); and these are the invariable proportions which in this particular case unite with each other, so that either iron or sulphur may be left in excess in the conversion of the mixture, according to the way in which it happens to have been made. Iron does indeed form another compound with sulphur—one atom of iron to two atoms of sulphur—but not under the conditions given. These two sulphides of iron (FeS and FeS_2) afford an illustration of the law that the elements combine with each other in simple proportions, or in simple multiple proportions. FeS it will be seen = 1 : 1, whilst FeS_2 = 1 : 2 atoms.

A good example of chemical change, in which the atomic relationships are easily arrived at, is furnished by passing hydrogen gas over heated oxide of copper. The hydrogen abstracts the oxygen from the oxide, leaving metallic copper and yielding water. The change is thus expressed by chemical notation, $CuO + H_2 = Cu + OH_2$; and, if the weight of the atoms entering into this chemical change be substituted for the symbolic statement, we have 79·5 : 2 :: 63·5 : 18, and this relation is invariable for this particular change. Here then is another example of the law of definite proportions (Dalton's law).

Another important chemical law (generally known as the law of Gay-Lussac) is the following :—'Whenever in a chemical reaction two or more of the factors are gaseous, or capable of existing in the state of vapour, it is found that the definite proportions observed in the reaction are either the proportions of the vapour or gas densities or else some simple multiple of these proportions.' For instance, hydrogen combines with oxygen in the following way :—Two atoms of hydrogen plus one atom of oxygen give water ($H_2 + O = H_2O$). Or taking the atomic weights, 2 + 16 = 18; then as

Hydrogen required.		Water formed.		Density of hydrogen.		Density of steam.
2	:	18	::	1	:	9

and the ratio 2 : 18 is the ratio of the molecular weight of hydrogen to the molecular weight of water.

The terms molecule and atom have been much employed in a confused sense, and have at times been taken as synonymous, but modern

chemistry, as we have seen, attaches distinct meanings to these words. We have already defined a molecule as the smallest mass into which a substance is susceptible of division without changing its chemical nature. Supposing that a lump of sugar (a substance which contains the elements carbon, hydrogen, and oxygen) be pulverized to the utmost, the smallest particle so obtained, even if it be microscopic, has still the qualities of sugar, and so it would be if the division were continued down to the molecule. If the sugar be dissolved in water we obtain a subdivision which, we may believe, extends as far as the molecules, but on evaporating the solution the sugar can be recovered in its solid condition, and as the qualities of the sugar are present the molecules have remained intact. Now, although the molecules are the limit of the physical subdivision of a substance, the chemist carries the subdivision still further; but the products then obtained no longer possess the qualities of the original substance, and one or more new substances result. If the sugar of the preceding experiment be exposed to heat it melts, browns, blackens and gives off gases and vapours of a pungent character, and finally carbonizes into a black mass of charcoal, which, although bulky, weighs much less than the original sugar. The molecules have therefore been resolved into charcoal and gaseous products, and the constituent atoms have been torn apart and caused to figure in fresh arrangements. The carbon atoms are partly in the charcoal and have been partly dispersed in the gaseous products, and the hydrogen and oxygen have gone off likewise in various forms of combination, one of them being water.

Since all substances may be resolved by chemical processes into one or more of the seventy or so elementary bodies, it is plain that all substances not themselves elementary are to be regarded as resulting from the union of two or more elementary substances. We may now take the law of definite proportion into closer consideration, for it applies to all kinds of chemical change and to all classes of substances, elementary as well as compound. Elementary substances are plainly susceptible only of changes in the direction of building up compounds: when they combine, however, the union takes place in definite proportions, by weight or volume. There is the important supplement to the law of definite proportions, namely, the law of multiple proportions, and if we accept the atomic theory, both laws are necessary consequences of the constitution of matter which this theory assumes to exist.

There are five compounds of nitrogen with oxygen, viz.:—

	Formula.	Contains N by weight.	Contains O by weight.	Ratio.
Nitrous oxide	N_2O	28	16	2 : 1
Nitric oxide	N_2O_2	28	32	2 : 2
Nitrous anhydride	N_2O_3	28	48	2 : 3
Nitric peroxide	N_2O_4	28	64	2 : 4
Nitric anhydride	N_2O_5	28	80	2 : 5

Here we have at once an exemplification of the law of definite and of multiple proportions, and these proportions are invariable for each of the particular substances specified.

ATOMS AND MOLECULES OF VARIOUS SUBSTANCES.

	Formula.	Weight of atoms.	Weight of molecule.
Carbonic anhydride	CO_2	12 + 32	44[1]
Chloride of sulphur	SCl_2	32 + 71	103
Sulphide of iron	FeS	56 + 32	88
Ammonia gas	NH_3	14 + 3	17
Sulphuretted hydrogen	SH_2	32 + 2	34
Calcium carbonate	$CaCO_3$	40 + 12 + 48	100
Sodium chloride	NaCl	23 + 35·5	58·5

We can always deduce from the definite proportions, discovered from the chemical compounds a substance forms, a value which is either its molecular weight or else some simple multiple of this quantity, and in most cases the principles of chemical science enable one to decide which of the different values is the true molecular weight. Let us take alcohol as an illustration: it is found by direct experiment that the specific gravity of alcohol vapour referred to hydrogen gas is twenty-three, then since by Avogadro's law equal volumes of alcohol vapour and hydrogen gas under the same conditions have an equal number of molecules, the molecule of alcohol is twenty-three times as heavy as the molecule of hydrogen, and as the molecule of hydrogen contains two atoms of hydrogen the alcohol molecule weighs forty-six times the weight of one atom of hydrogen.

Alcohol (C_2H_6O) has the following constitution:—

	Atomic weights.
C_2 =	24
H_2 =	6
O =	16
	46

[1] Forty-four times the weight of hydrogen molecule H_2.

which expressed as a percentage gives the following relationship :—

Carbon	52·18
Hydrogen	13·04
Oxygen	34·78
	100·00

Sufficient use has already been made of symbols and formulae to show that the facts of chemistry are expressible in symbolic language, and the expressions range from very simple statements to exceedingly complex ones. The following facts will further elucidate the employment of the simpler symbolical statements.

The substance *lime* is a compound of calcium and oxygen, the molecule containing one atom of each ; the formula is, therefore, CaO. If ordinary lime be slaked with water there is the familiar development of heat, and a new substance is formed as follows :—

$$CaO + H_2O = CaH_2O_2, \text{ or } Ca(HO)_2.$$

The new substance is called calcium hydrate. In this reaction two molecules unite to form a third. Calcium hydrate is fairly soluble in water, and a clear solution can be obtained by filtering the milky liquid. If carbonic anhydride be passed into this liquid it becomes turbid from the formation of a new substance (calcium carbonate). The reaction is thus expressed :—

$$CaH_2O_2 + CO_2 = CaCO_3 + H_2O.$$

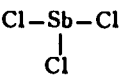
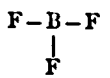
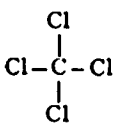
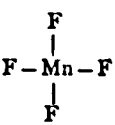
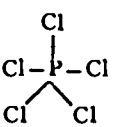
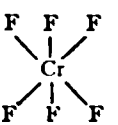
The new substance is practically chalk, and, if it be separated and an acid such as hydrochloric acid be poured upon it, disengagement of the carbonic anhydride takes place, and a fresh compound of calcium is formed thus :—

$$CaCO_3 + 2HCl = CaCl_2 + CO_2 + H_2O.$$

The new substance is calcium chloride, and we can discern what the other product accompanying the CO_2 is. Carbonic acid is familiar to us as the gas formed during alcoholic fermentation, in which case it is produced by the chemical decomposition of sugar, as we shall see further on. The atoms of different substances have not the same power of replacement when interchange takes place ; for instance, one atom of sodium is able to displace one atom of hydrogen in an acid, and so sodium chloride (NaCl) is formed when sodium is brought into conjunction with hydrochloric acid. Calcium, on the other hand,

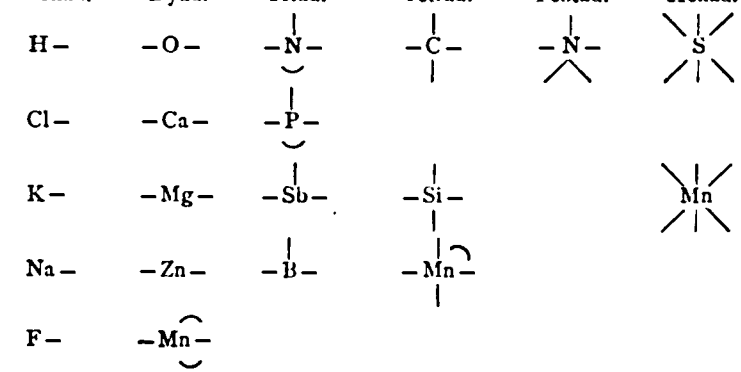
can displace two atoms of hydrogen, forming $CaCl_2$ as in the above equation. In the case of sulphuric acid (H_2SO_4), which has two displaceable atoms of hydrogen, two atoms of sodium are required to furnish sodium sulphate (Na_2SO_4), whilst only one atom of calcium is needed to give calcium sulphate ($CaSO_4$). This relationship of the chemical atoms is called their *valency*, and we speak of elements as monovalent, divalent, trivalent, &c., the terms being usually shortened into monads, dyads, triads, tetrads, pentads, &c. The valency of an atom is shown not only by its capacity for replacing other atoms, but also by its power of uniting with other atoms, or what is also termed its atom-fixing power.

EXAMPLES.

		Expressed graphically.
(1) Sodium chloride	NaCl	Na–Cl
Sodium fluoride	NaF	Na–F
(2) Calcium chloride	$CaCl_2$	Cl–Ca–Cl
Calcium fluoride	CaF_2	F–Ca–F
(3) Antimony chloride	$SbCl_3$	
Boron fluoride	BF_3	
(4) Carbon tetrachloride	CCl_4	
Manganese tetrafluoride	MnF_4	
(5) Phosphorous pentachloride	PCl_5	
(6) Chromium hexafluoride	CrF_6	

The explanation which modern chemistry gives is this: each of the elementary atoms is assumed to have a definite number of bonds, and by these it can be united to other atoms. The compounds just enumerated afford an illustration of all the powers of attachment from one 'monad' valency to six or 'hexad' valency, and some other examples have been given earlier in this chapter indicating the values

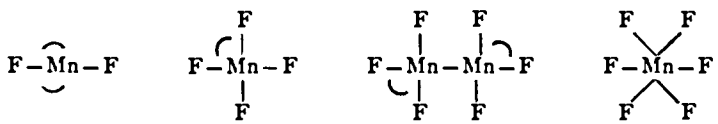
of hydrogen and oxygen. The valency of atoms of the elements, taken alone, may be exemplified thus:—



In symbolic notation the same idea is conveyed by the use of dashes and Roman numerals placed above the symbol to the right, thus:—

$$H' \quad Zn'' \quad C^{iv} \quad N^{v} \quad S^{vi}$$

If the valency exceed dyad value the higher valencies may be suppressed in pairs, so that a hexad atom may figure as a tetrad or dyad, and a pentad may exhibit triadic or monadic valency; the pair of bonds suppressed is indicated by a small curve. The fluorides of manganese give an excellent example of varying valency in the same elementary substance:—



In carbonic anhydride all the bonds of the carbon are combined with oxygen (O = C = O); in carbonic oxide a pair of attachments is suppressed (C = O).

Compounds of only two elements are termed binary compounds. When the same element forms two compounds with oxygen, the termination *-ic* is retained for the higher oxide, whilst the termination *-ous* is given to the lower, thus:—ferrous oxide (FeO), ferric oxide (Fe_2O_3). The names of binary compounds of other elements are formed like those of the oxide.

Compounds of chlorine are called chlorides.

Compounds of sulphur are called sulphides.

Of compounds of two or more elements the most prominent class is that of the acids, a term originally applied only to substances possessing a sour taste like vinegar, but analogy has necessitated a wider use of the term. An acid may be defined as a compound containing one or more atoms of hydrogen, which become displaced by a metal or by the hydrate of a metal. An acid containing one such atom of hydrogen is said to be monobasic, two such atoms dibasic, &c. If the same element forms two acids with oxygen, the acid containing the larger amount of oxygen receives the terminal syllable *-ic*, whilst that containing less oxygen is made to end in *-ous*; thus we have phosphorous acid and phosphoric acid, sulphurous and sulphuric acids.

The term anhydride, or anhydrous acid, is applied to the residue obtained by the abstraction (in combination with oxygen as water) of all the displaceable hydrogen from one or two molecules of an oxygen acid, thus:—

$$H_2SO_4 - H_2O = SO_3 \text{ (sulphuric anhydride).}$$
$$2HNO_3 - H_2O = N_2O_5 \text{ (nitric anhydride).}$$

The anhydride is capable of recombining with water to form the corresponding acid again.

In every acid it is possible by chemical processes to replace the hydrogen wholly or partially by different metallic elements, hydrates, oxides, &c., and thus obtain a large quantity of compounds, called *salts*. Elements or groups of elements so displacing the hydrogen are termed *bases*. Good examples of bases are:—sodium oxide, Na_2O; lime, CaO; zinc oxide, ZnO; sodium hydrate, $NaHO$; potassium hydrate, KHO; ammonia, NH_3.

If the salt be free from oxygen and sulphur, like common salt, $NaCl$, it is termed a haloid salt: if it contain oxygen it is termed an 'oxysalt,' and if this oxygen be replaced by sulphur it is known as a 'sulphosalt.'

Oxysalts are divided into normal, acid, and basic:—

A normal salt is one in which the displaceable hydrogen of the acid is all exchanged for an equivalent amount of a metal or of a positive compound radical. Example:—sodium nitrate, $NaNO_3$ from HNO_3; calcium sulphate, $Ca''SO_4$ from H_2SO_4; potassium phosphate, K_3PO_4 from H_3PO_4.

An acid salt is one in which the displaceable hydrogen of the acid is only partially exchanged for a metal or positive compound radical:—

Sulphuric acid, H_2SO_4; hydric sodic sulphate, $NaHSO_4$.

Carbonic acid, H_2CO_3; hydric potassic carbonate, $KHCO_3$.

Phosphoric acid, H_3PO_4 { hydric disodic phosphate, Na_2HPO_4. dihydric sodic phosphate, NaH_2PO_4.

When the number of bonds of the metal or compound positive radical contained in a salt exceeds the number of atoms of displaceable hydrogen in the acid the compound is usually termed a basic salt, as, for instance:—

Carbonic acid, H_2CO_3; malachite, $Cu''_2H_2CO_3$.

The generic name of the salts of each acid is formed by changing the termination *-ic* into -ate, or the termination *-ous* into -ite, for instance:—

Sulphurous acid	forms	sulphites.
Sulphuric acid	„	sulphates.
Carbonic acid	„	carbonates.
Nitrous acid	„	nitrites.
Nitric acid	„	nitrates.

There is an important class of compounds, already spoken of in a general way, which bears the same relationship to water which salts hold to their respective acids. They are called 'hydrates,' and may be regarded as derived from water by replacing one-half of its hydrogen, thus:—

Potassium hydrate, KOH, from HOH (H_2O).
Calcium hydrate, CaO_2H_2, from 2HOH.
Silicic hydrate, SiO_4H_4, from 4HOH.

Compound Radicals.

The term 'compound radical' was applied by Liebig to any group of two or more atoms which takes the place and performs the functions of an element in a chemical compound; in practice, however, it is only applied to any such group occurring in numerous chemical compounds. An element may be regarded as a simple radical. A polyad element is essential to every compound radical, and, in fact, a compound radical consists of one or more atoms of a polyad element in which one or more bonds are unsatisfied, and it is either a monad, dyad, triad, &c. radical, according to the number of monad atoms required to satisfy its active atomicity. Such a radical cannot exist with its active chemical affinity unsatisfied, and so when isolated it combines with kindred radicals, forming a group in a state of chemical equilibrium. An almost infinite number of such bodies must exist,

for in the compounds of every polyad element it is only necessary to vacate successive bonds to create each time a new compound radical. Thus marsh gas (methane) CH_4, minus one atom of hydrogen, gives the compound radical methyl, CH_3; minus two atoms of hydrogen it forms methylene (CH_2''), and by the abstraction of three hydrogen atoms it is transformed into the triad radical formyl $(CH)'''$. For general convenience, no compound radical should be recognized unless it can be shown to enter into the composition of a large number of compounds.

Formerly the question as to how the elementary substances were arranged, united, or grouped in a compound was hardly raised, but now the manner in which the atoms are grouped together in the molecule has become an all-important question, and the ascertaining of the definite structure of the molecule is perhaps the chief object of modern chemistry. The considerations relating to the special relationship of the atoms of a compound constitute a new branch of chemical science called stereo-chemistry, and we shall gather some idea of the importance of this line of research in connexion with the problems of the changes induced in the sugars, &c., by fermentation, &c.

Chemistry of the Carbon Compounds.

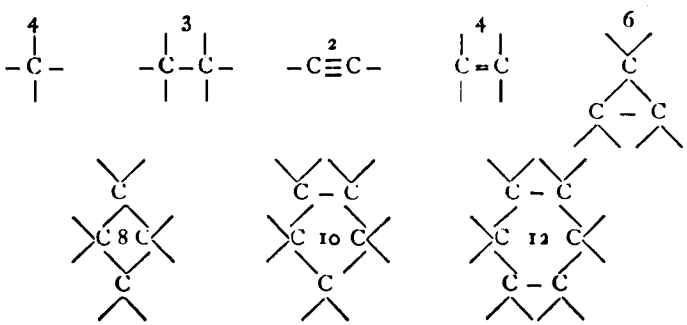
We may now direct our attention to the general nature of the carbon compounds more especially. The number of known compounds of this one element is far greater than that of all the compounds of other elements put together. The carbon compounds exhibit a great diversity in their molecular structure, which is often highly complex. As a rule, organic compounds consist of a very few chemical elements, but the number of atoms united in a single molecule may be very large, exceeding even 100. Carbon is more peculiarly the element of the organic world, but nitrogen plays also a part of the first importance in relation to living things or organized life, and hydrogen and oxygen have their share. It was formerly believed that the so-called organic substances were the outcome of vital processes only; but although the cause which determines the growth of organized beings is shrouded in mystery, we know that the materials of which they consist are subject to the same laws as mineral matter, and that the complexity may be traced to the peculiar attributes of carbon. Many of the organic compounds have been formed in the laboratory by purely chemical processes, and although

the chemist has never succeeded—and may never succeed—in forming a single organic cell, with its power of growth and development, he has reason to expect that at no very distant date he will be able to prepare artificially the materials of which the cell is fashioned and the various products which it elaborates during its life-history.

The important point in dealing with complex organic compounds is to discover, if possible, how the atoms are grouped, and this knowledge has led in many cases to the intentional synthetical formation of particular compounds.

As a rule, chemical combination only takes place readily between dissimilar atoms, but the one characteristic of carbon, on which the great complexity and variety of its compounds depend, is the power which its atoms possess of combining amongst themselves to an almost indefinite extent.

These groups of carbon atoms are of great stability, and they have been to a certain extent exemplified in the graphic formulae already adduced. A few more examples may be added, and it will easily be recognized that a great number of variations is possible in the valency



of these by varying the number of bonds linking the carbon atoms together. [The small numbers appended to each group show the active valency of the group.]

The determination of the composition of the carbon compounds depends mainly on the conversion of the carbon into carbonic anhydride and the hydrogen into water. Nitrogen is estimated either in the gaseous form or as ammonia, whilst the oxygen is in most cases estimated by difference. The chemical operation by which these results are mostly secured is called a combustion, and that kind of chemical analysis known as gas analysis not unfrequently plays an important part in the analysis of organic substances.

The empirical formula[1] of an organic substance is arrived at by determining the amounts of the elements present, say, carbon, hydrogen, and nitrogen, and calculating them as a percentage on the amount of substance taken; the percentage numbers are then divided by the atomic weight of the elements to which they refer and afterwards reduced to simple ratios. By the 'combustion' of acetic acid 100 parts yield—

Carbon	39·96 ÷ 12 = 3·33	}	= 1 : 2 : 1
Hydrogen	6·74 ÷ 1 = 6·74		
Oxygen by difference	53·30 ÷ 16 = 3·33		
	100·00		

The empirical formula corresponding to this is CH_2O, and the atomic weight of this expression is $12+2+16=30$. But the vapour density of acetic acid shows that its molecule weighs thirty times the weight of the molecule of H and sixty times the weight of the half-molecule or atom of hydrogen, we therefore assign $C_2H_4O_2$ as representing the molecule of acetic acid. It is generally agreed to consider the volume occupied by two parts by weight of hydrogen at any temperature and under any pressure as two volumes, and so the formulae which represent the number of parts by weight of the substances to which they refer, and which occupy in the gaseous state the volume of two parts by weight of hydrogen under like conditions of temperature and pressure, may conveniently be termed two-volume formulae. The two-volume formula of acetic acid is then $C_2H_4O_2$, but may be better expressed as $HC_2H_3O_2$, inasmuch as it is a monobasic acid, i. e. having one atom of hydrogen replaceable by an atom of a monad element, or a monovalent compound radical. Examples: potassic acetate, $KC_2H_3O_2$; ethylic acetate or acetic ether, $(C_2H_5)\,C_2H_3O_2$.

A second and more developed rational formula for acetic acid is C_2H_3OH, which has reference to reactions in which the group OH (hydroxyl) is replaced by a monad element, as in the following case:—

Acetic acid		Phosphorous pentachloride		Acetyl chloride
C_2H_3OH	+	PCl_5	=	$C_2H_3OCl + HCl + POCl_3$, or (CH_3COCl).

By certain means one atom of hydrogen in marsh gas or methane

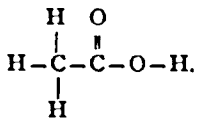
[1] That obtained by practice as apart from theory.

(CH_4) may be replaced by iodine, forming methylic iodide (CH_3I), which by the action of potassium cyanide is converted into methylic cyanide, $CH_3(CN)$. If this body be heated with water in the presence of acids or alkalies it is resolved into acetic acid and ammonia. Conformably with this mode of formation the formula of acetic acid may be written as in the following equation :—

$$CH_3CN + 2\,OH_2 = CH_3CO_2H + NH_3.$$

Lastly, by reason of the reaction with phosphorous pentachloride the group CO_2H is resolved into CO(OH), so that the formula becomes $CH_3CO(OH)$, and it is found that all the reactions in which acetic acid takes part are capable of representation by this formula.

We then write sodic acetate as $CH_3CO(NaO)$ or CH_3CO_2Na, but not $CH_2NaCO(OH)$, because it is found that when acted on by PCl_5, NaO is removed and replaced by Cl, just as HO is in acetic acid. We thus see how evidence is obtained as to the internal composition of a chemical compound, but the rational exposition must not be regarded as having any absolute significance. It is a convenient way of expressing a certain relationship, or the chemical history of the compound. The graphic formula for acetic acid, or that showing the respective valencies, is



A remarkable phenomenon is not unfrequently exhibited amongst organic compounds, which, although not unknown in the mineral kingdom, is peculiarly characteristic of the carbon compounds. There are a large number of cases where substances of the same chemical composition and the same vapour density have nevertheless distinctly different properties. They are called *isomeric* compounds[1]. Butyric acid and acetic ether provide an illustration. Analysis of each gives the formula $C_4H_8O_2$, and as the vapour densities are the same, it follows that the molecules of these two substances contain the same number of atoms of the same three elements, carbon, hydrogen, and oxygen. Acetic ether has a not unpleasant smell, somewhat resembling apples, whilst butyric acid has the highly un-

[1] Bodies of the same percentage composition, but of different vapour densities, are termed polymeric.

pleasant smell of rancid butter. The cause of this difference must lie in the atomic grouping or structure of the compounds. Now these two isomers when split up by chemical agents behave very differently, and a study of the resulting reactions leads one to infer that certain groups of atoms (compound radicals) are present in the compounds, because it is known that they exist in the products which these compounds respectively yield. Our knowledge of the structure of these very radicals depends on yet other reactions, by which they may be resolved into simpler groups. Thus, for instance, if we act on acetic ether with potassic hydrate two products are obtained, viz. potassic acetate and ordinary or ethylic alcohol. Alcohol has the formula C_2H_5HO and contains the radical C_2H_5, which is called ethyl. Further, we know that potassic acetate has the formula (graphically expressed) $K-O-(C_2H_3O)$ and contains the radical C_2H_3O, which is known as acetyl. We therefore infer that acetic ether contains both of these groups, and its simple formula of $C_4H_8O_2$ becomes the more graphic exposition, $C_2H_5-O-C_2H_3O$. The reaction then between this body and potassium hydrate consists of a simple interchange between the potassium and the radical C_2H_5, thus :—

Acetic ether		Potass. hyd.		Potass. acetate		Alcohol
$C_2H_5-O-C_2H_3O$	+	$K-O-H$	=	$K-O-C_2H_3O$	+	C_2H_5HO.

Taking the radical ethyl into consideration we can show that it may be formed in a compound which contains the radical CH_3 (methyl), by replacing one of the hydrogen atoms of this radical by another group of the atoms CH_3, thus :—

First methyl compound		Second methyl compound		Ethyl compound		Hydrogen compound
$H-C(H)(H)-x$	+	$H-C(H)-y$	=	$H-C(H)(H)-C(H)(H)-x$	+	$H-y$

(x and y are used instead of writing the formulae of definite compounds). The structure of acetyl can be worked out in a similar manner to that of ethyl, and in this case will be :—

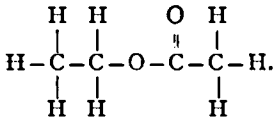
Ethyl

$$H-C(H)(H)-C(H)(H)-$$

Acetyl

$$-C(=O)-C(H)(H)-H.$$

Hence we conclude that the structure of a molecule of acetic ether should be

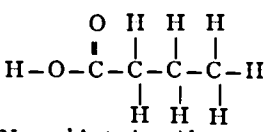


If butyric acid, the isomer of the body just spoken of, be acted on with potassium hydrate we obtain wholly different products; they are potassic butyrate and water, and the reaction is interpreted as follows:—

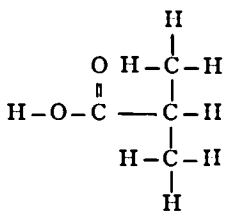
Butyric acid + Potassic butyrate

$$H-O-C_4H_7O + K-O-H = K-O-C_4H_7O + H-O-H.$$

It appears then that butyric acid, instead of containing the two radicals C_2H_5 and C_2H_3O, contains the more complex radical C_4H_7O and the simple radical H, but the reaction gives no information as regards the grouping of the atoms in the radical. An investigation of the structure reveals the fact that there are two different radicals having the same atomic composition, and corresponding to two distinct varieties of butyric acid which differ in their odour, their boiling point, and other qualities; and further, various reactions show that the atoms of the radicals are arranged in the two acids as expressed by the following formulae:—



(Normal butyric acid prepared from butter or by butyric ferentation)



(Isobutyric acid produced synthetically)

There are at least three substances having the composition $C_4H_8O_2$.

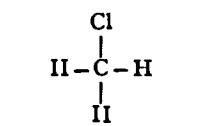
One of the most characteristic differences between carbon and the other elements is its capability of uniting with hydrogen, in various definite proportions, to form a great variety of compounds termed *hydrocartons*. These bodies are of especial interest from a theoretical point of view, since all carbon compounds may be considered as derived from them, either directly or indirectly.

The simplest known hydrocarbon is methane or marsh gas, whose composition is expressed by the formula CH_4. It is the first or lowest term of a series of hydrocarbons of gradually increasing complexity which are expressed by the general formula C_nH_{2n+2}, and are called the paraffins. The first members of the series are—

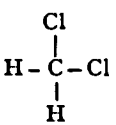
Methane (marsh gas) . . .	CH_4
Ethane	C_2H_6
Propane	C_3H_8
Butane	C_4H_{10}
Pentane	C_5H_{12}
Hexane	C_6H_{14}
&c.	

It will be discerned that between each successive term in this series there is a constant difference of CH_2. Bodies which stand in such a constant relationship are termed homologous (*ὁμός*, like, *λόγος*, a word).

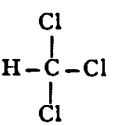
The substances enumerated range from a gas, through liquids to solids. Crude petroleum contains many members of the series, hence their name of paraffins. The paraffins are *saturated*[1] compounds; they cannot enter directly into combination. It is possible, however, to remove hydrogen from them, and thus enable them to combine directly with such elements as chlorine, bromine, and iodine (the halogens). The hydrogen atoms in methane can, for instance, be successively removed by chlorine and the following four bodies obtained:—



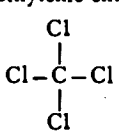
Monochloromethane
(methylic chloride)



Dichloromethane
(methylenic chloride)



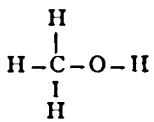
Trichloromethane
(chloroform)



Tetrachloromethane
(carbon tetrachloride)

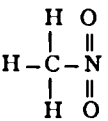
[1] When compounds contain no carbon atoms which are linked to each other by more than one bond, they are termed *saturated* compounds. If, however, they contain one or more pairs of atoms linked they are said to be *unsaturated*.

The monovalent group (H – O –) may displace an atom of hydrogen, thus:—



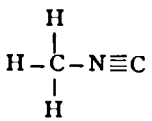
(Methylic alcohol)

and the compound radicals may do the same thing. NO_2, $O=\underset{|}{N}=O$ taking the place of hydrogen in methane gives—



(Nitromethane or methyl nitrite)

CN (cyanogen) C≡N – substituting hydrogen in CH_4 gives cyanomethane (CH_3CN)



belonging to the class called cyanoparaffins.

The Olefines.

Besides the paraffins which are saturated compounds there are other series of hydrocarbons which are unsaturated, i. e. the number of hydrogen atoms in each member of the series falls short of that which is possible if the carbon atoms are combined to their fullest extent: thus there is the olefine or ethylene series with the general formula CnH2n. Since methylene CH_2 apparently cannot exist, the series begins with ethylene C_2H_4, and the members which follow are:—

Propylene . .	C_3H_6
Butylene . . .	C_4H_8
Amylene . . .	C_5H_{10}
Hexylene. . .	C_6H_{12}
&c.	

The olefines may be regarded as derived from the corresponding paraffins (CnH2n + 2 series) by the removal of two atoms of hydrogen:

this is effected by the action of dehydrating agents such as sulphuric acid, phosphoric anhydride, and zincic chloride on the alcohols of the ethylic series, thus $CnH_{2n+1}OH = CnH_{2n} + OH_2$.

Terpenes,

or hydrocarbons of the CnH_{2n-4} series. Those of the composition $C_{10}H_{16}$ are chiefly known; they include the greater number of the essential or volatile oils (for instance, hop oil). The terpenes from natural sources exhibit the power, to a greater or less extent, of causing the deviation of a ray of polarized light either to the right or to the left.

The Alcohols.

The term *alcohol* is of Arabic origin, and was originally applied specifically to the volatile inflammable liquid which is the characteristic product of the fermentation of sugar. Since then, however, it has been extended to a class of bodies which for the most part possess properties more or less analogous to those of spirit of wine or ethylic alcohol. A still further extension of the term has been made to those compounds of carbon, hydrogen, and oxygen, which may be regarded as derived from a hydrocarbon by the displacement of one or more hydrogen atoms by an equal number of the monad group OH, thus including such substances as glycerin [1], mannite, phenol, &c. The alcohols bear a certain relationship to the metallic hydroxides or hydrates. Thus the OH is replaceable by acid radicals; for example, just as KOH yields KCl, $KHSO_4$, $KC_2H_3O_2$, so methyl alcohol CH_3OH yields CH_3Cl, CH_3HSO_4, $CH_3C_2H_3O_2$. We will confine our attention to the class of alcohols containing one OH group: monohydric alcohols and those of the $CnH_{2n+1}OH$ or ethylic series, for this class contains the best known alcohols, viz. those that are associated with fermentative processes. We may mention the following:—

		Boiling point.
Methylic alcohol	$CH_3.OH$	66° C.
Ethylic alcohol	$C_2H_5.OH$	78° C.
Propylic alcohol	$C_3H_7.OH$	97° C.
Butylic alcohol	$C_4H_9.OH$	108° C.
Amylic alcohol	$C_5H_{11}OH$	131° C.

[1] Glycerin is a polyhydric alcohol derived from propane (C_3H_8), having the formula, $C_3H_5(OH)_3$.

It will be seen how these bodies correspond with the C_nH_{2n+2} series of hydrocarbons. Primary alcohols yield on oxidation, first an aldehyde and then an acid, both of which contain the same number of carbon atoms as the alcohol.

Acetaldehyde

$$C_2H_5OH + O = C_2H_4O + OH_2.$$

Acetic acid

$$C_2H_4O + O = C_2H_4O_2.$$

The Ethers.

The ethers are a class of compounds bearing the same relation to the alcohols that the metallic oxides bear to their hydrates. Thus $K_2O + H_2O = 2KOH$, and by suitable means the following reaction can be obtained:—

Ethyl oxide — Ethylic alcohol
Ethylic ether — Ethylic hydrate

$$(C_2H_5)_2O + H_2O = 2\,C_2H_5OH$$

We are only concerned with the ethers corresponding to the $(C_nH_{2n+1})_2O$ series, as being those more especially met with in fermentation.

Ordinary ether (ethylic ether) may be prepared by heating ethylic alcohol with concentrated sulphuric acid at 140° to 145° F. with suitable precautions. The reaction takes place in two parts, and is typical of a class of reactions by which the ethers are produced from the corresponding alcohols.

Hydric ethylic sulphate (or sulphovinic acid)

$$(1)\ C_2H_5OH + H_2SO_4 = C_2H_5HSO_4 + OH_2.$$

Ethylic ether

$$(2)\ C_2H_5OH + C_2H_5HSO_4 = (C_2H_5)_2O + H_2SO_4.$$

This is a cyclical action in which the H_2SO_4 is regenerated, and, theoretically, the same quantity of acid should convert an unlimited quantity of alcohol into ether; but subsidiary reactions, and dilution by the water formed in the reaction, prevent this continuity.

Amylic alcohol (fusel oil) treated in the same way yields amylic ether. A mixture of alcohols produces mixed ethers. The following ethers correspond to the alcohols of the C_nH_{2n+1} series.

			Boiling point.
Methylic ether	C_2H_6O	$\left\{\begin{matrix}CH_3\\CH_3\end{matrix}\right\}O$	$-21°$ C.
Ethylic ether	$C_4H_{10}O$	$\left\{\begin{matrix}C_2H_5\\C_2H_5\end{matrix}\right\}O$	$35{\cdot}5°$ C.
Propylic ether	$C_6H_{14}O$	$\left\{\begin{matrix}C_3H_7\\C_3H_7\end{matrix}\right\}O$	$85°$–$86°$ C.
Butylic ether	$C_8H_{18}O$	$\left\{\begin{matrix}C_4H_9\\C_4H_9\end{matrix}\right\}O$	$141°$ C.
Isoamylic ether	$C_{10}H_{22}O$	$\left\{\begin{matrix}C_5H_{11}\\C_5H_{11}\end{matrix}\right\}O$	$176°$ C.

The ethers of this series are, as a rule, colourless liquids having an agreeable odour—very often fruity—sparingly soluble or else insoluble in water. By prolonged heating with water they are resolved into the alcohols which originally gave rise to them, for example:—

$$\left\{\begin{matrix}C_2H_5\\C_2H_5\end{matrix}\right\}O + OH_2 = 2\,C_2H_6O.$$

The Aldehydes.

The term *aldehyde*, which is an abbreviation of alcohol dehydrogenatum, was originally assigned by Liebig to the compound C_2H_4O, which may be regarded as alcohol less two atoms of hydrogen. The term is now applied to all compounds containing the group COH (formyl) as, from the manner in which aldehyde is formed from alcohol and from its reactions, there can be no doubt that the CH_2OH group of the alcohol (CH_3CH_2OH) is alone affected and is changed by the withdrawal of two atoms of hydrogen into the group COH.

Formic aldehyde, or formaldehyde HCOH (methyl aldehyde), is produced very readily by the moderate oxidation of the vapour of methylic alcohol:—$CH_3OH + O = HCOH + OH_2$. Formic aldehyde is an antiseptic, and is employed under the name of formalin in milk, &c.

Ordinary or acetic aldehyde (ethaldehyde) belongs to the class $CnH2n+1\,COH$, or acetic series: it is the product of nearly all oxidizing agents on ethylic alcohol; thus

$$CH_3CH_2OH + O = CH_3COH + OH_2.$$

It is a colourless, very mobile, inflammable liquid, possessing a somewhat pungent but not unpleasant smell. It is doubtless produced in small quantities during fermentation.

The aldehydes heated with nascent hydrogen yield the corresponding alcohols, thus :—

$$CH_3COH + H_2 = CH_3CH_2OH.$$

Acetic aldehyde is converted into acetic acid by the further action of oxidizing agents :—

$$CH_3COH + O = CH_3COOH.$$

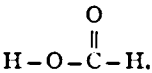
The aldehydes may be regarded as a class of compounds intermediate between the primary alcohols and the corresponding acids.

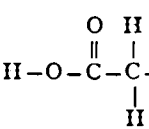
The Acids.

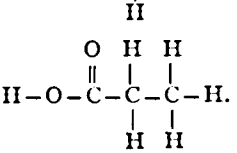
Organic acids are formed from the alcohols by oxidation, and the primary alcohols yield a corresponding series. The most probable explanation of the reaction which takes place is afforded by the assumption that the CH_2OH group has undergone conversion into the COOH or *carboxyl* group, so that the organic acids are represented by rational formulae containing the group COOH.

One of the most characteristic properties of the organic acids is that of forming metallic salts when acted on by metallic carbonates, the change consisting in all cases of the removal of hydrogen and its replacement by the equivalent amount of the metal. The number of atoms of hydrogen thus replaced gives the basicity of the acid, which is in direct relation to the number of times that the group COOH is contained in its rational formula.

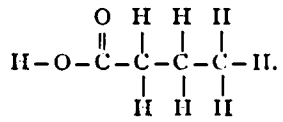
The organic acids which are of interest from their connexion with fermentation are the monobasic acids of the acetic and lactic series. The acetic series $C_nH_{2n+1}COOH$ furnishes, amongst others, the following members :—

Formic acid . $\begin{cases} H \\ COOH \end{cases}$ 

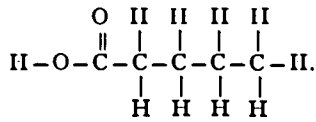
Acetic acid . $\begin{cases} CH_3 \\ COOH \end{cases}$ 

Propionic acid $\begin{cases} C_2H_5 \\ COOH \end{cases}$ 

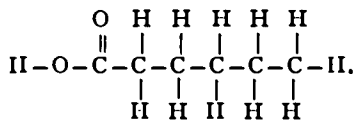
Butyric acids . $\left\{\begin{matrix}C_3H_7\\COOH\end{matrix}\right.$



Valeric acids . $\left\{\begin{matrix}C_4H_9\\COOH\end{matrix}\right.$



Caproic acids . $\left\{\begin{matrix}C_5H_{11}\\COOH\end{matrix}\right.$



&c.

Many of these acids are met with in nature; some are produced by the action of yeast in fermentation under conditions which are hardly the normal ones.

Acetic acid, the most familiar of those enumerated, is produced from ethylic alcohol. The ordinary process of vinegar-making is dependent on the oxidation of ethylic alcohol by the intervention of an organism which oxidizes the alcohol at the expense of the oxygen of the air. The reaction corresponding to the various methods of preparation is in two stages, thus:—

(1) $CH_3CH_2OH + O = CH_3COH + H_2O$.
(2) $CH_3COH + O = CH_3COOH$.

A typical method for the preparation of an organic acid from the corresponding alcohol is to submit the latter to the action of hydrobromic or hydriodic acid, after which the resulting bromide or iodide is converted into cyanide by digesting with potassium cyanide. The cyanogen compound is finally decomposed by heating with water and a mineral acid, or an alkali:—

(1) $\underset{\text{Alcohol}}{C_2H_5OH} + \underset{\text{Hydriodic acid}}{HI} = \underset{\text{Ethylic iodide}}{C_2H_5I} + OH_2$.

(2) $\underset{\text{Potas. cyanide}}{C_2H_5I} + KCN = \underset{\text{Ethylic cyanide}}{C_2H_5CN} + \underset{\text{Potas. iodide}}{KI}$.

(3) $C_2H_5CN + 2OH_2 = \left\{\begin{matrix}C_2H_5\\COOH\end{matrix}\right. \underset{\text{Propionic acid}}{} + \underset{\text{Ammonia}}{NH_3}$.

The acid obtained by this method has, it will be observed, one

unit of carbon more than the alcohol employed. Methylic alcohol by the same series of reactions would yield acetic acid.

Butyric acid is produced, together with acetic and caproic acids, by the bacterial fermentation of lactic acid. In a pure state it is a colourless liquid, having an odour resembling acetic acid, but turning to a rancid odour when volatilized or diluted. It is perhaps the isomer of butyric acid, or some other acid of this series, which possesses one of the most characteristically unpleasant odours known.

Isobutyric acid occurs, together with formic, acetic, propionic, and benzoic acids, in the banana. Butyric acid, like acetic and lactic acids, is the product of a definite group of bacterial organisms—the butyric ferments so called.

Lactic Acid, $CH_3.CH(OH)COOH$,

is formed from various carbohydrates, sugars, starches, &c., under favouring conditions of temperature. The acids of this series are monobasic, and have the general formula CnH2n(OH)COOH. They bear the same relation to the acids of the acetic series that the monohydric alcohols of the ethylic series bear to the paraffins, hence they are termed monobasic dihydric acids.

Carbonic acid is sometimes regarded as the first term of the lactic series of acids, $H_2CO_3 = CO(OH)_2$, being the monohydroxyl derivative of formic acid, HCO(OH), the first term of the acetic series. It is, however, a dibasic acid.

By heating the acids of the lactic series with a concentrated aqueous solution of hydriodic acid they are reduced to the corresponding acids of the acetic series.

(1) CnH2n(OH)COOH + HI = CnH2n I COOH + OH_2.
(2) CnH2nCOOH + HI = CnH2n + 1 COOH + I_2.

Lactic acid is a strong, odourless acid forming a large class of salts, viz. lactates. We shall have occasion to allude to both lactic and butyric acids again in the practical technological portion of this book.

General Conceptions of Chemical Science.

The general tendency of natural chemical change is to produce substances of more stable equilibrium. The falling back from a less stable to a more stable condition is always attended by the evolution of heat (as, for instance, in the decomposition of cane sugar by yeast

into the familiar products of fermentation), whether the change results from the spontaneous decomposition of a single complex compound, or from the concurrence of several decompositions in a chemical reaction. Since action and reaction are always equal and opposite, it may be confidently assumed that through whatever series of chemical changes these unstable products may have been formed, an equal amount of heat or an equivalent amount of some other form of energy must have, for the time being, disappeared.

In all cases of chemical change the tendency is to form those products whose formation will determine the greatest evolution of heat, and if we were dealing only with disassociated atoms, their union to form molecules would in every case be attended by the evolution of heat, and the different amounts evolved would be all positive quantities and all directly comparable. But beginning with molecules of elementary compounds, the formation of other molecules may or may not be attended by the evolution of heat, and often heat is absorbed. Heat is required to part the atoms in a molecule, and the resulting thermal effect will depend on whether the amount of heat absorbed at the parting is greater or less than that evolved by the new union. We thus come to classify chemical compounds into *exothermous*, whose heat of formation is a positive quantity, and *endothermous*, whose heat of formation is negative; and chemical reactions are spoken of in the same sense as being exothermic (in which heat is evolved) and endothermic (in which it is absorbed). We arrive at the following generalizations :—

1. The heat absorbed in the decomposition of a substance is equal to the heat evolved in its formation, provided the initial and the final states are the same.

2. The heat evolved in a series of successive chemical changes is equal to the sum of the quantities which would be evolved in each separately, provided the final conditions are the same.

3. In any series of chemical changes, when the initial and final conditions are the same, the total thermal effect is the same, however different the processes by which the results may be reached.

CHAPTER VI

THE CARBOHYDRATES

IT would seem desirable that the substances concerned in alcoholic fermentation and yeast nutrition should be taken into closer consideration. This entails the study of three classes of substances: (1) the carbohydrates, which embrace as members the starches, the gums allied to the starches, and the sugars; (2) the albuminoids and allied nitrogenous organic substances, termed amides; (3) the mineral or inorganic substances which have to do with the vital economy of the yeast cell. We will endeavour to deal concisely, but adequately, with the two more important classes—the carbohydrates and the albuminoids—and then take all three groups in their relationship to the yeast cell.

The name carbohydrates is applied to a large class of substances which are of great importance in their relationship to living things, being the chief constituents of plants, and playing a considerable part in their association with animal tissues.

The carbohydrates are allied to the polyhydric alcohols (arabitol, mannitol, and dulcitol), and consist of carbon, oxygen, and hydrogen, the last two elements being present in the proportions in which they form water; so that the general formula can be expressed as $Cn(OH_2)m$, and it will be seen that the members of this family with which we shall deal accord with this expression.

A marked difference exists in one of the physical attributes of the carbohydrates which enables us to group them under two main divisions: (1) those which are diffusible (crystalloids), e.g. the sugars; (2) those which are not diffusible[1] (colloids), e.g. starch and the starch gums.

[1] Like many other classifications this division is hardly absolute, as there exist carbohydrates which are on the border line, being difficult but not impossible to diffuse.

The Sugars.

The more important subdivisions of this group are as follows:—

	Simple formula.	Examples.
The pentose sugars . .	$C_5H_{10}O_5$.	Arabinose, xylose.
The hexose or glucose group (or glycoses)	$C_6H_{12}O_6$.	Glucose, fructose.
The saccharon group or di-saccharides	$C_{12}H_{22}O_{11}$.	Cane sugar, maltose, lactose.
The trisaccharides . . .	$C_{18}H_{32}O_{16}$.	Raffinose.

The Starches and Starch Congeners.

(Other names—amyloids, saccharo-colloids, polysaccharides.)

Cellulose
Dextrin
Glycogen
Inulin
α and β Amylan
Starch

General formula $(C_6H_{10}O_5)n$ { All probably large molecules. Starch a very large molecule.

For the purposes of chemical analysis, the carbohydrates are mainly distinguished by the following characteristics:—

1. Speaking generally, their solutions are optically active, that is to say, they have the power of rotating or twisting a ray of polarized light. If this twisting takes place in the direction in which the hands of a clock move, the solution is said to be dextro-rotary, if in the opposite direction, laevo-rotary: the first-named readings have the + sign attached to them, and the last named the − sign. The instrument used for obtaining analytical data of this kind is termed a 'polarimeter.'

The same weight of the same carbohydrate under identical conditions always exhibits the same power of rotation of polarized light, but the amount of rotation varies with the particular carbohydrate under examination, and this fact affords a valuable means of distinguishing between them, and even, under given circumstances, of estimating the quantity of the optically active substance present in solution.

The specific rotary power is the amount of rotation expressed in degrees of arc calculated on 10 grams of a substance, contained in 100 c.c., considered as viewed in a layer of 1 metre (1000 mm.) in depth.

Observations with the polarimeter are usually made in tubes of either 100 mm. or 200 mm. in length, and some polarimeters read directly in degrees of arc, whilst others are graduated to arbitrary scales. The most convenient instrument for general use by brewers,

distillers, &c. is what is called a half-shadow instrument, designed to be used with a gas or paraffin flame and graduated in degrees of arc. Values obtained with such sources of illumination are termed AJ values, whilst those obtained with the monochromatic sodium flame are distinguished by the sign AD. A practical point of no little importance in polarimetric observations is that the solution should be brilliantly clear, and this can be effected either (1) by adding a little powdered asbestos before filtering, or (2) by adding some alumina cream to the liquid, allowing to settle and then filtering. If the solution is highly coloured, it must be diluted till it can conveniently be read in the 100 mm. tube. The addition of a little sodium sulphite sometimes helps very much: avoid decolourizing with animal charcoal if it can possibly be dispensed with.

2. Another property which most of the sugars possess is of much service to the chemist: it is the power of precipitating cuprous oxide (Cu_2O) from a solution containing alkaline tartrate of copper (Fehling's solution): this property is most conveniently expressed by speaking of the amount of cupric oxide (CuO) yielded by one gram of a reducing sugar. Every sugar that is capable of reducing Fehling's solution does it to an extent which, under similar conditions, is invariable. This method of analysis is of so much importance that we may briefly outline it here. Thirty c.c. of Fehling's solution (see Appendix) are taken and mixed, in a beaker, with 60 c.c. of well-boiled boiling distilled water, then put into a water-bath in which the water is boiling. A few c.c. of the solution to be analysed are measured out and introduced into the beaker with stirring. Leave in the boiling-water bath for twelve minutes, then filter straightway, and wash with hot boiled water till any blue colouration is practically removed from the filter-paper and the washings are colourless. Dry the paper containing the Cu_2O and burn in a porcelain crucible, chopping up the pieces carefully with a spatula. On heating, the Cu_2O becomes oxidized to CuO, thus $Cu_2O + O = 2CuO$. It is advisable not to use more of the saccharine solution than will give from 0·16 to 0·2 grams of CuO, that is to say, the Fehling solution must be in fair excess so that the filtrate is a full blue colour. If the method be worked on these lines and with precaution, results agreeing within ·0015 gram can be obtained. The volumetric method for estimating sugars with Fehling's solution may be regarded as practically useless, except where the merest approximations to accuracy are aimed at.

The solution value, or specific gravity in solution, of the carbo-

hydrates is such that one divisor pretty nearly meets all cases. One gram of cane sugar dissolved in distilled water and contained in 100 c.c. of solution weighs 100·386 grams, or, taking a volume of such a solution equal to 1000 grains of water at 60° F., it will weigh 1003·86 grains: the number above 1000 divided by 3·86 gives the grams per 100 c.c., or practically the percentage, since 1 c.c. of water weighs 1 gram. This divisor, 3·86, holds for cane sugar, glucose, fructose, and some others, but the products of the transformation of starch by diastase, viz. maltose, dextrin, and malto-dextrin, have a higher solution value and require the divisor 3·95, that is to say, that a volume equal to 1000 grains of distilled water at 60° F. containing 1 gram of these carbohydrates in solution weighs 1003·95 grains. In researches on the carbohydrates it is by no means unusual to employ one divisor throughout. Neither divisor is accurate for all possible concentrations of solutions of the substances, but is sufficiently near for practical purposes.

The Pentose Sugars

are closely allied to the hexoses or glycoses. Only a few substances of this group have been separated and identified, and they are not of great importance from the point of view of the fermentation industries; they are, however, not without interest. We may mention two. First, the sugar arabinose derived from gum arabic by the action of dilute acid on the pentosan constituent araban, and, secondly, the substance xylose produced from the pentosan substance xylan by the same means. Tollens obtained the two pentose sugars in this way from brewer's grains: the chemical change is most probably the following:—

Pentosan Pentose

$$C_5H_8O_4 + OH_2 = C_5H_{10}O_5.$$

Malt contains, it is said, the whole of the pentosans which were present in the barley from which it was made.

The pentosans are also called 'furfuroids,' as they yield the substance 'furfurol' by distillation with a 12 per cent. solution of hydrochloric acid. A pentose is first formed, and the subsequent reaction is

Pentose Furfurol

$$C_5H_{10}O_5 = C_5H_4O_2 + 3OH_2.$$

Furfurol is a substance of strong smell and flavour, and the presence of any appreciable quantity in a fermented liquid would undoubtedly be a material disadvantage.

The pentose sugars are crystalline substances, not susceptible of fermentation under ordinary conditions.

Arabinose is believed to be the aldehyde of a pentahydric alcohol (arabitol, $C_5H_{12}O_5$). It reduces Fehling's solution. Its specific rotary power = 106·5 AD.

The Hexose Sugars.

Dextrose, glucose or d glucose, grape sugar is found in great abundance in the vegetable kingdom, as, for instance, in ripe sweet fruits, such as grapes, plums, figs, &c., which, in addition to glucose, contain a considerable amount of fructose (levulose) and small quantities of cane sugar. Glucose is met with also in the stems of the cereals and in the flowers of many plants from which bees extract honey. It is found in small quantities in the seeds of cereals such as barley, and this quantity is increased by germination. Glucose is prepared in large quantities as an article of commerce by acting on the starch of cereals such as maize, rice, &c., with dilute acid, usually sulphuric acid. The chief steps in the manufacture of glucose from cereal starch are the following:—Disintegration of the raw material; boiling under pressure with dilute acid, neutralizing the acid; concentration in vacuo; decolourization; further concentration; solidification, and finally breaking up into convenient-sized lumps. Although in such a process there is considerable likelihood of other substances being produced—indeed, their production is sometimes aimed at—yet the action can be so regulated that glucose is the main product. The action is termed a 'hydrolizing' one, that is, one in which water is caused to enter into chemical combination with the carbohydrate substance. If we express starch by its empirical formula, then the formation of glucose takes place thus:—

$$C_6H_{10}O_5 + OH_2 = \underset{\text{Glucose}}{C_6H_{12}O_6}.$$

Glucose is very readily fermentable by most of the Saccharomycetes. It reduces Cu_2O from Fehling's solution, yielding 2·31 grams of CuO per 1 gram of glucose. The reducing power of glucose was formerly taken at 100, but the more accurate assessment of its reducing value has disturbed the old relationships with other reducing bodies. Glucose is readily soluble in water, and has a decidedly sweet taste, but not nearly as sweet as cane sugar weight for weight. Prepared in a pure crystalline state it carries one molecule of water of crystallization,

so the more accurate formula is $C_6H_{12}O_6,OH_2$. The specific rotary power of anhydrous glucose is 52·8 AD.

One of the most interesting, and perhaps the most important of the chemical reactions which yield glucose as one of the products, is the so-called inversion of cane sugar, which can be accomplished either by the action of dilute acids at temperatures approaching to boiling, or by the action of yeast at a temperature of 130° F. The degree of heat applied in the latter case is too high to permit of any fermentative action, but is favourable to the action of the active principle, invertase, which practically all yeasts contain. By either of these means cane sugar in solution can be completely inverted into glucose and fructose (dextrose and levulose), which are produced in equal quantities. The reaction is thus expressed:—

Cane sugar		Glucose		Fructose
$C_{12}H_{22}O_{11} + OH_2$	=	$C_6H_{12}O_6$	+	$C_6H_{12}O_6$.

And 100 parts of cane sugar yield 105·1 parts of invert sugar. The change is described as an inversion because the dextro-rotary cane sugar is changed into a mixture having a markedly laevo-rotary action.

Fructose, d Fructose[1], Levulose.

This substance, which has been just alluded to, may now be more particularly described: it is met with pretty abundantly in nature, and has its origin—as glucose doubtless has—in cane sugar. Fructose can be prepared in a fairly pure state from inulin, a carbohydrate substance which is closely related to starch, and which is contained in the tubers of the dahlia, the Jerusalem artichoke, &c. Heated with dilute acid inulin yields fructose very much in the same way as starch yields glucose, thus:—

Inulin		Fructose
$(C_6H_{10}O_5)n + OH_2$	=	$C_6H_{12}O_6$.

Fructose can only be obtained in the crystalline state with some difficulty; indeed, it is the presence of fructose in invert sugar which retards or prevents crystallization, and it may be looked upon as forming the uncrystallizable constituent, the visible crystals in samples of invert sugar being those of glucose. Fructose can, however, be obtained in silky needles. It is strongly laevo-rotary at ordinary

[1] The *d* does not indicate 'dextro-rotary,' but the chemical descent of the sugar. See p. 90.

temperatures. At 60° F. its specific rotary power is −95·6 AD, and invert sugar is laevo-rotary because the laevo-rotation of fructose is greater than the dextro-rotation of glucose. Fructose reduces Fehling's solution to a somewhat less extent than glucose, for 1 gram = 2·205 grams CuO. It is, like glucose, readily fermentable and diffusible, but in a solution containing glucose and fructose the glucose constituent ferments at a greater rate than the laevo-rotary sugar. Fructose is much sweeter than glucose; it is, indeed, as sweet as cane sugar.

Chemical relationship of Glucose and Fructose.

Now, although glucose and fructose proceed in equal quantities from the inversion or hydrolysis of cane sugar, and have the same empirical formula, they are not of the close chemical relationship that might be supposed, and are not true optical antipodes. Glucose, as shown by Kiliani, belongs to a class of substances termed the aldoses, which are bodies of an aldehydic nature containing the group COH. The pentose sugars belong to the same classification.

The extended formula for d glucose, which is also that for other hexose sugars, galactose and mannose, is

$$CH_2OH.4CHOH.COH = C_6H_{12}O_6.$$

The sugar fructose, on the other hand, is called a 'ketose,' being closely related to a class of substances termed the ketones, which may be looked upon as derivatives of the aldehydes in which the H atom of the COH group is replaced by a compound radical derived from a 'paraffin.' For example, when the H atom in aldehyde is replaced by CH_3 (methyl), acetone is formed containing the group CO (carbonyl).

Aldehyde Acetone

$$CH_3-COH \text{ gives } CH_3-CO-CH_3.$$

The ketoses stand in the same relation to the ketones that the aldoses do to the aldehydes.

The extended formula for d fructose is the following:—

$$CH_2OH-3CHOH-CO-CH_2OH = C_6H_{12}O_6.$$

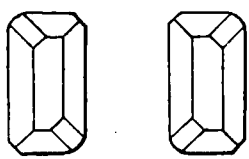
The hexahydric alcohols (mannitol, &c.) give rise by oxidation to aldoses and ketoses, forming the bodies known as the hexoses; these latter in turn yield di-, tri-, and polysaccharides by elimination of water.

When an aldose, or a ketose in aqueous solution, is heated for several hours on the water-bath with phenyl-hydrazine acetate, a somewhat

complicated reaction ensues, which has for one result the production of a compound containing one molecule of the sugar with two molcculcs of phenyl-hydrazine. Glucose under these conditions yields glucosazone. Fructose yields the same body, thus displaying the close relationship between the two sugars. The so-called osazones are usually crystallizable substances differing from each other, according to the source, in solubility, melting point, &c., and much use has been made of the differing properties in researches on the sugars. When heated with strong hydrochloric acid the osazones decompose, the phenyl-hydrazine part of the compound goes to the acid, whilst a substance termed an osone is left, and if this be subjected to the influence of nascent hydrogen evolved from zinc dust and acetic acid, two atoms of hydrogen are introduced into the osone. If glucosazone be treated in this way, fructose is obtained. The reaction is interesting and important, for by it we are able to transform an aldose into its corresponding ketose: this last, if optically active, always polarizes in the opposite direction to that of the aldose from which it was derived. The reverse process of transforming a ketose into its corresponding aldose is also a chemical possibility.

We have alluded several times to the optical activity of the carbohydrates, and given several specific values, from which it will perhaps have been inferred that the property of rotating a ray of polarized light is of very considerable importance as a means of research into the character of the optically active substances. The great chemist and physicist Pasteur was the pioneer in this branch of investigation, when nearly forty years ago he pointed out that optical activity was associated with crystalline form. Pasteur observed that substances which possess optical activity exhibit in their crystalline form a lack of symmetry of structure, and the steps which led to this remarkable discovery were these:—The substance to which his attention was directed was tartaric acid, which is obtainable from argol or wine-lees. Four modifications of this acid are now known. The tartaric acid from wine-lees, which is dextro-rotary, yields salts possessing the same property, but one of the modifications of tartaric acid, viz. racemic acid, is without action on polarized light and gives inactive compounds. The crystals of these two acids are, on a superficial examination, identical, and were for a long time considered to be so, but Pasteur detected a difference which had escaped the observation of others. He found that, in the case of the optically active tartaric acid, small facets were discernible on the crystals, and these facets were so arranged that the crystals

were unsymmetrical or oppositely symmetrical, differing from each other, in a manner, as the right hand differs from the left. The crystals of racemic acid, on the contrary, are symmetrical, their mirror image coinciding in space with themselves. Pasteur's attention was, at the same time, directed to quartz crystals, which were known to exist in right and left-handed forms—some of the crystals being dextro-rotary and others laevo-rotary as regards their action on polarized light. In the case of quartz these facts had already been linked together, and Pasteur was led to believe that the rotary power of tartaric acid was due to its unsymmetrical molecular constitution.



DEXTRO & LAEVO AMMONIUM BIMALATE. FIG. 7.

On preparing the sodium-ammonium salts of tartaric and racemic acids respectively, Pasteur found that the racemate deposited unsymmetrical crystals, but one-half of them proved to be oppositely symmetrical to the other half, the facets being to the right and left respectively; the one form was therefore the mirror image of the other. (A good example of this opposite symmetry is ammonium bimalate).

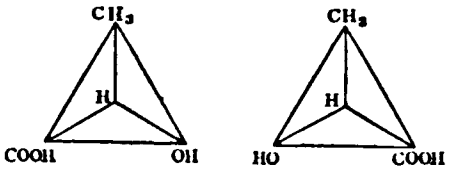
Pasteur next separated the two enantiomorphous[1] forms, and on making a solution of each discovered the important fact that the right-handed crystals were derived from ordinary dextro-rotary tartaric acid, whilst the left-handed crystals were derived from a, till then, unknown acid, having an equal but opposite rotary power to the dextro-acid. The new acid was called laevo-tartaric acid, and so racemic acid was proved to consist of equal amounts of dextro- and laevo-tartaric acid, which compensate or neutralize the optical effect of one another.

Most optically active compounds are capable of existing in at least three forms, viz. dextro-rotary, laevo-rotary, and inactive, or what is now termed a racemoid modification. These forms are designated by the letters d, l, and i, written in front of the substance, as d glucose, but in the cases of the alcohols and acids the letters refer not to their own rotation, but to that of the aldehydes to which they are related.

It was soon found that the ordinary chemical formulae were inadequate to represent these optical differences, as they do not present the disposition of the atoms in space. A new departure in chemical cience has resulted from the attempts to show the disposition of atoms in the molecule of a substance in the three dimensions of space,

[1] The scientific term used to describe such cases.

a branch of chemical science known as stereo-chemistry. The hypothesis by which Le Bel and Van 't Hoff's names have become famous has led to the most important results. They assume that the atom of carbon and its affinities may be represented by a regular tetrahedron, the carbon atom being at the centre, its four affinities being represented by the four corners of the geometrical figure. Thus the following arrangement represents the two lactic acids.



DEXTRO & LAEVO-LACTIC ACIDS.

FIG. 8.

(Inactive lactic acid consists of equal parts of these two substances.) To such a carbon atom, which is capable of having its four attached groups arranged round it in two different ways, Le Bel and Van 't Hoff gave the name *asymmetric*, the one necessary condition for such a state of things being that all the four attached groups must be different. Such an arrangement causes the tetrahedrons to be non-superimposable in space, that is to say, they cannot be made to match each other in the positions , but if one of the groups in each tetrahedron be replaced so as to give two similar groups in each (e.g. if the OH group in Fig. 8 be replaced by H: see Fig. 9), then it is found that the tetrahedrons can be superimposed. (This fact is rendered much more plainly evident by making small models of the tetrahedrons of paraffin wax, &c., marking the poles or summits and putting them into juxtaposition).

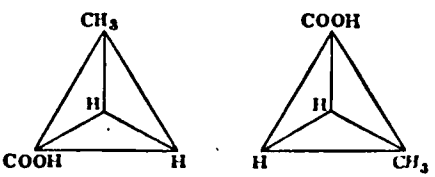


FIG. 9.

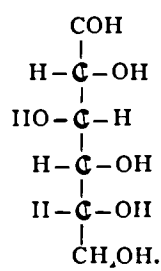
It is a curious fact, but susceptible of explanation, that the ordinary optically active substances are obtained as the result of vital processes, but whenever a substance containing an asymmetric carbom atom is prepared artificially it is found to be inactive to polarized light. In the cases of natural production some selective influence seems to be at work: in the case of the artificial elaboration of the substance there

is no more reason why one isomer should be formed than the other, so that in the production of an infinite number of, say, lactic acid molecules, there is an equal probability of both forms being produced, and—one may assume—an equal production of dextro- and laevo-rotary molecules leading to an inactive substance: so in the artificial production of tartaric acid, racemic acid is formed. A very interesting mode of separation of the components of inactive combinations is by submitting them to selective bacterial decomposition. Professor P. Frankland some years ago found an organism which could decompose laevo-rotary glyceric acid and leave the dextro-rotary isomer untouched. Another bacterium can attack the laevo-rotary component of inactive lactic acid. This matter of selective fermentation becomes, perhaps, more interesting when one comes to the sugars. Emil Fischer, who by synthetical processes has managed to build up many sugars, including the true optical antipodes of dextrose and levulose, has obtained amongst them one which is only distinguishable from the well-known levulose (d fructose) by the circumstance of its being optically inactive. To this inactive artificial sugar he gave the name of fructose. On fermenting it with brewery yeast Emil Fischer found that only one-half of it was decomposed with the formation of alcohol and carbonic anhydride, and that the unfermented portion was now as strongly dextro-rotary as levulose is laevo-rotary; therefore the fermentable portion corresponded to ordinary levulose, or, as it is now generally termed, d fructose.

It follows from the hypothesis expressed just now that the sugars, as optically active substances, must be represented as containing asymmetric carbon atoms. The graphic formulae for d glucose and d fructose stand thus, taking into account the asymmetric carbon atoms, four in the first and three in the second, which are printed in different type:

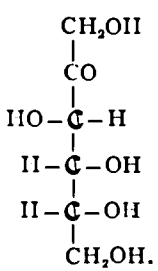
d Glucose

$CH_2(OH)(CHOH)_4COH.$



d Fructose

$CH_2(OH).(CHOH)_3.CO.CH_2OH.$



Theory enables one to predict the existence of no less than sixteen active modifications of grape sugar and eight inactive. Most of these have been actually discovered. Enough has, perhaps, been said to show the great importance of stereo-chemistry in its relationship to the sugars, and to some of the more abstruse phenomena of fermentation: it may safely be predicted that extraordinary results will follow from the pursuit of this branch of scientific investigation within the next few years. The fermentation of sugars of the hexose group, as a general statement, takes place according to the equation:—

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2;$$

which gives theoretically

For crystallized glucose .	$C_6H_{12}O_6OH_2$.	46·46 per cent. of alcohol,	
For anhydrous glucose .	$C_6H_{12}O_6$.	51·10	„ „

but inasmuch as about 5 per cent. of by-products, chiefly glycerin and succinic acid, are formed in the general way,

100 parts anhyd. glucose yield 48.5 parts by weight of alcohol.

The Disaccharides or Saccharoses.

Cane sugar (sucrose, $C_{12}H_{22}O_{11}$) is the most familiar substance in this particular group of sugars. Its frequent occurrence in nature, and the important part it plays in the economy of living organisms, give it a peculiar interest, as also does its susceptibility to the action of the yeast organism. Cane sugar is found in all parts of plants—root, stem, leaves, fruit, seed, &c.—being formed during the vital processes. The sorghum cane contains about 15 per cent., the sugar cane 20 per cent., and the sugar beet 10 to 12 per cent of cane sugar.

Cane sugar is readily fermentable, but the fermentation is preceded by the inversion of the cane sugar into glucose and fructose (dextrose and levulose), as already mentioned, by the action of the enzyme invertin or invertase. This last substance exists in practically all the true Saccharomyces, and exerts what is termed a hydrolizing action on cane sugar.

Cane sugar does not reduce Fehling's solution, and is markedly dextro-rotary, the specific rotary power being AD 66·5.

When cane sugar is fermented by yeast, unless a large excess of the ferment be employed, or some nitrogenous yeast nutrient be added, such as yeast water, asparagin, &c., it is not easy to secure the fermentation of the whole of the sugar even in rather weak solutions (say 5 per cent.). In solutions exceeding 10 per cent. in strength the

considerable quantity of alcohol produced will exert an inhibitive effect which introduces a fresh factor of disturbance. Formerly it was thought that the decomposition of sugar by yeast was attended by the production of alcohol and carbonic anhydride simply, but Pasteur showed that only about 95 per cent. is decomposed in this way, the remainder being accounted for by the presence in the fermented liquid of succinic acid and glycerin, and the actual loss of a small amount which goes to form cell tissue.

According to Pasteur, 100 parts of sugar (glucose) give the following general results:—

Alcohol	48·4
Carbonic anhydride . .	46·6
Glycerin	3·3
Succinic acid	0·6
Cellulose	1·2
	100·1

One hundred parts of cane sugar yield on inversion 105·2 parts of invert sugar (equal parts of glucose and fructose), and so may be considered as consisting of a glucose base or residue and a fructose base, so that the yield of alcohol, making the above allowances for by-products, is equal to some 51·1 per cent. of the weight of cane sugar fermented. This matter of the by-products is of no little importance, as will be seen when we come to the practical aspects of the alcoholic-fermentation industries.

Maltose, $C_{12}H_{22}O_{11}$.

Another important example of the disaccharide sugars calling for more than a passing description is the sugar produced when starch in a gelatinized state is submitted to the action of certain enzymes, such as the active substance diastase which is furnished by malt. It is from its presence in malt, but more especially from the production of a considerable quantity of maltose when malt is mashed by the brewer and distiller, that the sugar takes its name. Maltose was rediscovered[1] by C. O'Sullivan in 1872, who described its properties and instituted a series of researches into the conditions under which it is formed, and opened up a prolific field of investigation into which other chemists were not slow to enter. Maltose is one of the earlier products of the action of dilute acids upon starch, an action

[1] A previous discovery by two other chemists had been made a good many years before, but the matter had been lost sight of.

which is much enhanced when conducted under pressure—generally of steam. The prolonged action of dilute acid converts the maltose into glucose, so that maltose may be regarded as consisting of two glucose residues associated in one molecule. Reverting to the action of diastase or gelatinized starch: if a thoroughly gelatinized[1] mass of potato starch be acted on at ordinary temperatures by a not inconsiderable quantity of a strong cold-water infusion of ground malt filtered bright, and the action be allowed to proceed for some twenty hours or so, the starch paste becomes limpid, and it is not difficult to demonstrate by chemical means that the greater part of the starch has been converted into the sugar maltose, which by suitable means could be separated and obtained as a white crystalline substance, having a marked power of reducing Fehling's solution and a strong dextro-rotary action on polarized light. Maltose has a somewhat sweet taste—much less sweet than glucose—and is readily soluble, like most of the other sugars, in water. It is fermentable by most yeasts, but not nearly so readily as glucose. C. O'Sullivan says[2], that if a mixture of sucro-dextrose (glucose) and amylon (maltose) be fermented with yeast, the glucose entirely disappears before the maltose is touched. Maltose was formerly believed to be directly fermentable, but the accepted view now is that it undergoes a prior conversion into glucose, owing to the intervention of an enzyme akin to glucase, which exerts a hydrolizing action similar to invertase, but yielding two molecules of glucose instead of invert sugar, thus:—

Maltose Glucose

$$C_{12}H_{22}O_{11} + OH_2 = 2C_6H_{12}O_6.$$

One gram of maltose yields with Fehling's solution 1·37 grams of CuO. The specific rotary power of the sugar is AD 138·6, and the mean divisor to obtain grams per 100 cc. from spec. grav. is 3·95. [1 gram of maltose per 100 cc. gives a solution of spec. grav. 1003·95.]

Raffinose, $C_{18}H_{32}O_{16} + 5OH_2$.

An instance of a trisaccharide sugar. It is found in molasses, in beetroot, and in the seeds of cereals. It is a crystalline substance soluble in water, and having a faintly sweet taste. It is dextro-rotary. and has no reducing power on Fehling's solution. When heated with

[1] See under Starch.

[2] Article on Carbohydrates, Miller's *Organic Chem.* (1880), part iii. p. 613.

dilute acid, it gives equal molecules of fructose and a disaccharide melibiose isomeric with lactose $C_{12}H_{22}O_{11}OH_2$ (milk sugar). Melibiose on prolonged treatment with a dilute acid splits up into galactose and glucose in equal molecules—

Raffinose Melibiose Fructose

(*a*) $C_{18}H_{32}O_{16} + OH_2 = C_{12}H_{22}O_{11} + C_6H_{12}O_6$

Galactose Glucose

(*b*) $C_{12}H_{22}O_{11} + OH_2 = C_6H_{12}O_6 + C_6H_{12}O_6$.

These reactions show that raffinose is a trisaccharide, i.e. that it contains 3 C_6 groups. Some yeasts can ferment raffinose, others partially invert it to fructose and melibiose, and ferment out the former substance, leaving the latter.

The Saccharo-colloids, or Polysaccharides.

(General formula $(C_6H_{10}O_5)n$.)

Cellulose, empirical formula $C_6H_{10}O_5$.

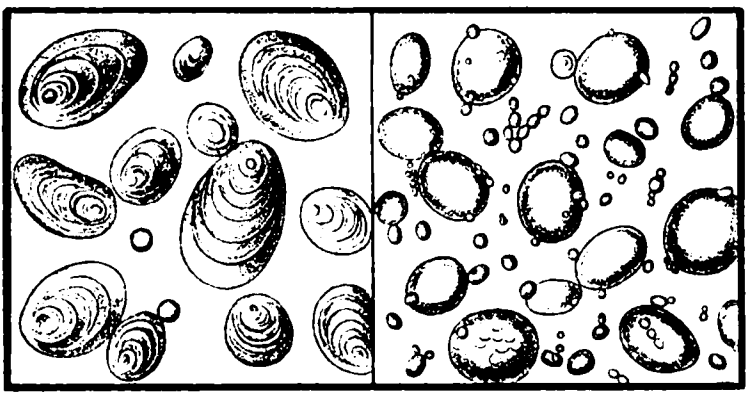
This substance forms the groundwork of the tissues of all plants, from the tallest tree to the most insignificant fungus. Fairly pure natural forms of cellulose exist in pith, cotton, the ivory-nut, &c. It is convenient to regard cellulose as existing in two forms in vegetable tissues (1) a very stable, resisting form, which is not altered easily by any chemical means, (2) a state in which moderately dilute acids acting under pressure can convert it into glucose: this latter form is termed hemicellulose or oxycellulose. The cellulose of a date-stone or the ivory-nut is a good example of the dense kind, whilst the cellulose of the inner compartments of the seeds of cereals and of leaves furnishes an example of the alterable form. Pure cellulose is a white semi-transparent colloidal substance, having a specific gravity of 1·45. The true chemical composition of cellulose is expressed by some multiple of $C_6H_{10}O_5$, but we do not know what the multiple is.

Starch.

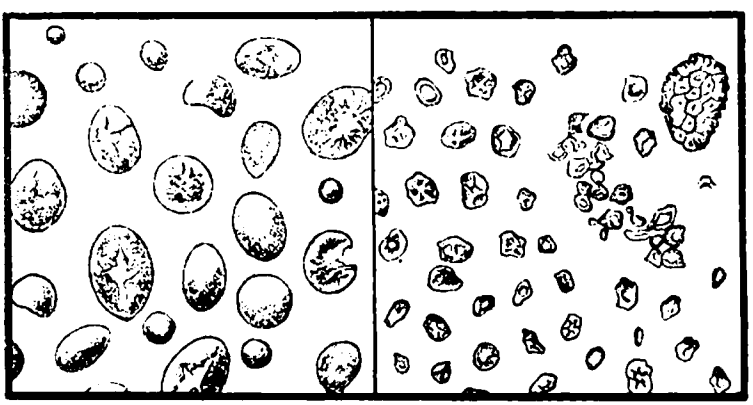
Formulae generally employed, $C_{12}H_{20}O_{10}$ or $C_{18}H_{30}O_{15}$.

Also known as amylum (Greek, ἄμυλον). A substance which is very widely spread throughout the vegetable kingdom. It is found in all the organs of plants, being a product of the assimilation of carbonic

Plate V.



Potato. *Wheat.*



Maize. *Rice.*

Starches.

(*After Matthews & Lott.*) x 350.

anhydride by leaves under the influence of sunlight. It not only functions as a material from which the needs of the growing plant are more or less continuously supplied, but constitutes a reserve nutrient material for later vegetative processes. Starch is a substance of especial interest and importance to the fermentation technologist or specialist, as it is the main source of the sugar which is to be converted wholly or partially into alcohol. It is to the cereals that he looks for the supply of the raw materials. Barley, which is the chief source of the malt used by the brewer and distiller, contains from 60–66 per cent. of starch, and nearly the whole of this can be made available in furnishing saccharine matters in the worts employed for fermentation.

Starch is deposited in the cells of plants in a granular state, and is formed in such a manner that the newest part of the granule is inside, not outside. Plate V gives examples of starches from different sources. The granules are not homogeneous in their nature—at all events as regards density and solubility—they appear to possess a kind of resisting envelope bearing some resemblance to cellulose in its nature. This envelope resists at ordinary temperature the action of the enzymes found in finished malts, though it breaks down easily in the growing plant or seed under the influence of more potent cell-dissolving agents. If once the envelope be ruptured or removed, the underlying and inner portions of the granule are susceptible of being acted on by the diastase contained in cold-water malt extract.

It may be remarked at this point that the chemistry of the transformation of gelatinized starch by diastase has attracted so many investigators, that the literature of the subject would fill a considerable-sized volume. It is only possible, in the space at our command, to indicate the more general lines on which the work has been conducted, and the more practical issues of it. Much has been done, but much remains to be done to completely elucidate the matter.

Brown and Heron[1] ascertained by direct experiment that the integrity of the outer coating of the starch granule is the cause of its being able to resist the action of diastase. They dealt with the matter as follows:—Starch in the granular state was triturated with quartz sand or powdered glass so as to bruise or break the granules, when it was found that in this state it was readily acted on by

[1] *Journ. Chem. Soc. Trans.*, 1879, pp. 624 et seq.

diastase even in the cold. Under favourable conditions of temperature the transformation of the starch could be carried to a very far point. An experiment is quoted in which the following proportions were obtained:—

Maltose . 97·8 per cent.
Dextrin . 2·6 per cent.

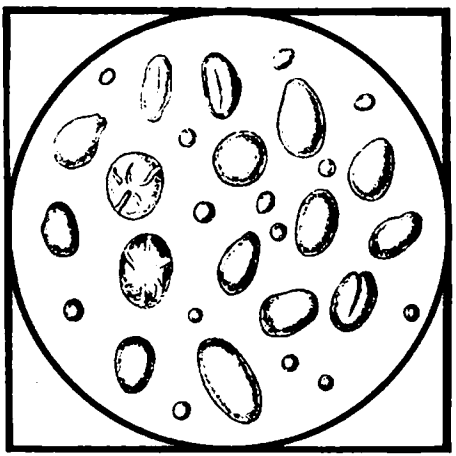
and this includes the small quantity of cellulose forming the resistant coating, which is probably converted into dextrin. One may say then that under the most favourable conditions, starch is almost wholly hydrolizable to maltose.

On suspending starch in a quantity of water, just sufficient to allow the mixture to be moved with a glass rod, and whilst so moving it running in boiling water, a viscid semi-transparent paste is formed and the starch is said to be gelatinized[1]. If the process of gelatinization be contrived to take place under the microscope, or made so slow that portions of the liquid can be taken out at intervals and thus examined, the granules are seen to swell up to a huge size (Plate VI), the edges then break and frill, and the large and almost shapeless masses that then form, spread away into a diffused amorphous condition. The granules behave rather like a gum during this change. Gelatinization paves the way to the ready action of malt diastase which, as we have said, cannot attack the perfect granule in the cold. There is nevertheless a small residue of some substance in malt which can attack the envelope of the starch granule at temperatures well below the ascertained temperature of gelatinization of barley starch, which is somewhere about 170° F. Having prepared a small quantity of well-gelatinized starch paste, say 100 c.c., and the temperature having fallen below 170° F., let us add to it 10 cc. of a cold-water infusion of ground malt[2] and trace the changes which ensue by taking out a drop or two of the solution at frequent intervals and testing them on a white porcelain surface with tincture of iodine or a solution of iodine in iodide of potassium. The following colour reactions are obtained:—

[1] About 5 grams of potato starch in a final volume of 100 c.c. gives a good thick paste for experiments.

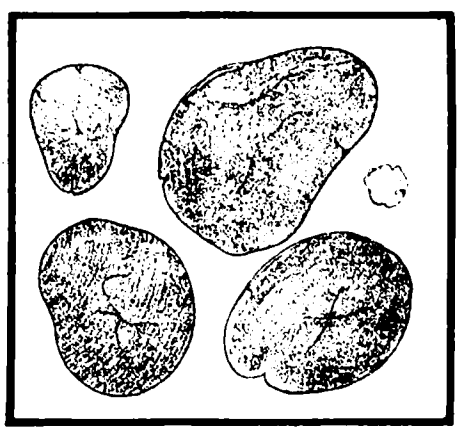
[2] Cold-water malt extract. 25 grams of malt flour may be infused in 75 c.c. of water at ordinary temperatures. Leave two hours and filter bright through a filter-paper.

PLATE VI.



Barley Starch.

× 350.



Starch granules gelatinising.

Reaction.	The solution contains
1. Blue (colour readily discharged by weak ammonia solution)	Gelatinized starch.
2. Blue „ „	Soluble starch.
3. Violet (not so readily discharged)	Erythro-dextrin[1].
4. Red brown „ „ „	Erythro-dextrin.
5. No reaction	Achroo-dextrins.

The production of these tints in succession, and their ultimate disappearance, mark chemical changes in the solution which are of the first importance in their relationship to the operations of brewing and distilling. The substances enumerated above are successively produced in the reaction, but besides these there are two very important ones. (1) The sugar maltose which is present in the solution after stage (2) is passed, and which continues to increase in quantity up to a limit determined by

(*a*) The quantity of malt extract (diastase) present;

(*b*) The temperature at which the reaction takes place or—what comes to the same thing—the temperature to which the malt extract has been exposed prior to employing it for the transformation of the starch.

(*c*) The time during which the action is allowed to proceed.

The other product is a peculiar association of the ordinarily fermentable substance maltose with the unfermentable body dextrin. In this condition the maltose is not fermentable by ordinary yeast. In the present state of knowledge it is convenient to term such substances malto-dcxtrins, and to regard them as products intermediate between maltose on the one hand and dextrin on the other. The characteristic property they possess is that they are sensitive to the action of cold-water malt extract, which is able to liberate the maltose portion and convert the dextrin portion into maltose, so that under these conditions the whole of the practically unfermentable malto-dextrin is converted into fermentable maltose. The two chief conditions which are favourable to the production of malto-dextrins are

(*a*) Restriction of the action of malt extract upon gelatinized starch in point of time;

(*b*) In point of temperature.

Malto-dextrins may be looked upon as representing a possible stage

[1] It is not altogether certain that this corresponds to a definite compound, as the colour may result from a mixture of 2 and 4. The author believes it does mark a separate compound.

in any starch transformation in which the maltose formed is not as yet liberated from combination with a dextrin which is simultaneously formed. The author has carried out experiments with Mr. C. Hyde Parker in which they have obtained bodies which correspond to the behaviour of malto-dextrins at all temperatures from 60° F. up to 150° F.

As a mode of general expression of the different stages in which the breaking down of starch by malt extract proceeds, we may take the following:—

	Stage i.	Stage ii.	Stage iii.	Stage iv.	Stage v.
Gelatinized Starch	Soluble starch	Maltose Erythro-dextrin* Malto-dextrin*	Maltose Erythro-dextrin* Malto-dextrin* Achroo-dextrin	Maltose Malto-dextrin* Achroo-dextrin	Maltose Dextrin (Furthest limit of the action of cold-water malt extract)

The bodies marked with a star are susceptible of being brought to the state of maltose by cold-water malt extract: indeed, erythro-dextrin or the erythro-dextrins are easily degradable into maltose. In this scheme the term achroo-dextrin is confined to non-reducing dextrin, or 'end' dextrin. Let us now consider how the changes just alluded to are to be represented by chemical notation.

C. O'Sullivan's[1] equation for starch transformations with cold-water malt extract for temperatures not exceeding 63° C. (145° F.) is as follows:—

$$\underset{\text{Starch.}}{C_{18}H_{30}O_{15}} + OH_2 = \underset{\text{Maltose.}}{C_{12}H_{22}O_{11}} + \underset{\text{Dextrin.}}{C_6H_{10}O_5}, \quad \text{or}$$

$$\underset{\text{Starch.}}{2\,C_{18}H_{30}O_{15}} + 2\,OH_2 = \underset{\text{Maltose.}}{2\,C_{12}H_{22}O_{11}} + \underset{\text{Dextrin.}}{C_{12}H_{20}O_{10}}$$

corresponds to:—

Maltose .	67·85 per cent.
Dextrin .	32·15 „
	100·00

The experiment was carried out on the basis of stopping the reaction at a point when a marked slackening in the action was observed; it being considered that the action takes place in two distinct stages, one a molecular change extending through the whole mass of gelatinized starch acted upon by the malt extract, and the second stage a gradual

[1] *Journ. Chem. Soc.* 1876, vol. xxx, p. 137.

hydrolizing of the substances produced till the limit of change was reached.

Brown and Heron[1] working under conditions that are not strictly comparable with C. O'Sullivan's experiments obtained the following equation for temperatures ranging from 60° F. to 140° F. In this equation starch is taken as $C_{12}H_{20}O_{10}$

Starch. Maltose. Dextrin.

$$10\,C_{12}H_{20}O_{10} + 8\,OH_2 = 8\,C_{12}H_{22}O_{11} + 4\,C_{12}H_{20}O_{10}$$

this corresponds to :—

Maltose . 80·9 per cent.
Dextrin . 19·1 ,,
100·0

Here the production of maltose is far greater than in C. O'Sullivan's corresponding equation, but it is fully accounted for by the fact that C. O'Sullivan, for reasons given above, did not allow the hydrolizing action to proceed so far.

The author in conjunction with Mr. Hyde Parker has recently carried out a number of starch transformations in which by a quite unintentional adjustment of the malt extract, transformations between temperatures of 60° F. and 150° F. during two hours were obtained giving proportions of maltose and dextrin exactly the mean of C. O'Sullivan's and Brown and Heron's results, viz.

Maltose . 74·3 per cent.
Dextrin . 25·7 ,,
100·0

In another series in which a larger amount of malt extract was employed the average results yielded between temperatures of 60° F. and 150° F. gave an amount of maltose a little higher than Brown and Heron's, so it is plain that the quantity of malt extract employed is an important factor in starch transformations.

Syniewski in a recently published research considers that the percentages

Maltose . 74·98
Dextrin . 25·02

obtained with starch transformations at the temperature of the laboratory mark the first stage of the hydrolizing action. The reaction stops almost completely at this stage. These figures agree with the mean of C. O'Sullivan's and Brown and Heron's results.

It is more than probable that there are two distinct phases of the

[1] *Journ. Chem. Soc. Trans.*, 1879, pp. 629 et seq.

action of cold-water malt extract, or, in other words, that it contains two kinds of diastase. One of these kinds operates in the direction of producing maltose, whilst the other yields dextrin. The effect of heat upon the active agents of the cold-water malt extract is to first impair the maltose-producing agent, and the last phase of the activity to be destroyed (this takes place at a temperature approximating to 170° F.) is the capacity for producing dextrin.

The complexity of the starch molecule is evidently very great to account for even the established molecular transformations, and a huge molecular value has been assigned by various investigators. For present practical purposes we may—taking Brown and Heron's view—consider the starch molecule (sometimes spoken of as the starch complex) as consisting of not less than ten groups of $C_{12}H_{20}O_{10}$ thus :—

$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$
$C_{12}H_{20}O_{10}$

The addition of OH_2 to each of these groups in succession leads to the production of successively increasing amounts of maltose, whilst the remaining groups at each of eight stages represent a compound dextrin susceptible of hydrolization until the limit is reached when two $C_{12}H_{20}O_{10}$ groups remain still unhydrolized and constitute so-called stable dextrin—an achroo-dextrin having no reducing power.

The first stage in the reaction would be thus expressed :—

Starch — Maltose — Erythro-dextrin i

$$10\,(C_{12}H_{20}O_{10}) + OH_2 = C_{12}H_{22}O_{11} + 9\,(C_{12}H_{20}O_{10})$$

The next stage would be :—

Erythro-dextrin i — Maltose — Erythro-dextrin ii

$$9\,(C_{12}H_{20}O_{10}) + OH_2 = C_{12}H_{22}O_{11} + 8\,(C_{12}H_{20}O_{10})$$

The third stage :—

Erythro-dextrin ii — Maltose — Achroo-dextrin i

$$8\,(C_{12}H_{20}O_{10}) + OH_2 = C_{12}H_{22}O_{11} + 7\,(C_{12}H_{20}O_{10})$$

and so on during the production of five more achroo-dextrins in turn. The final reaction would be expressible thus :—

Starch

$$10\,(C_{12}H_{20}O_{10}) + 8\,OH_2 = 8\,(C_{12}H_{22}O_{11}) + 2\,(C_{12}H_{20}O_{10})$$

the products being :—

Maltose . 80·8 per cent.
Dextrin . 19·2 „
100·0

It is difficult to obtain a production of maltose beyond this percentage from gelatinized starch.

A molecule of this size, breaking up as indicated, permits, it will be seen, of eight different dextrins, a number well in advance of those which have been satisfactorily identified. It is probable that as more is found out about the products of starch transformations, that a comparatively simple mode of expression will fulfil the requirements of the case. Before leaving the matter it is desirable to consider how the formation of malto-dextrin or malto-dextrins—regarding them for the moment as products differing from any of the dextrins already mentioned—may be represented by chemical notation.

The following expressions will serve:—

Starch Maltose Malto-dextrin

$$10\,(C_{12}H_{20}O_{10}) + 6OH_2 = 5\,(C_{12}H_{22}O_{11}) + \left\{ \begin{matrix} C_{12}H_{22}O_{11} \\ 2\,C_{12}H_{20}O_{10} \end{matrix} \right. + 2\,C_{12}H_{20}O_{10}$$

which corresponds with the following percentages:—

Maltose . .	51·07
Malto-dextrin	29·58
Dextrin . .	19·35
	100·00

Recent investigation renders it more than probable that the so-called malto-dextrins or amyloins are intermediate dextrins, and this has been practically proved in the case of one substance the malto-dextrin β of Ling and Baker and Eugen Prior's achroo-dextrin iii. The substances appear to be identical and each is nearly insoluble in 90 per cent. alcohol and is a yellowish-white powder having a slightly sweet taste. The specific rotary power is 171·1 and the reducing power 42·5 (maltose=100): these values are not far removed from those of Herzfeld's original malto-dextrin.

The facts so far as we have stated them lead one to the view that at a certain stage in the transformation a maltose molecule is formed within the complex and remains attached to the dextrin group from which it emanated, requiring the further action of diastase to free it from combination with the dextrin group. Very possibly this takes place only when the next group of $C_{12}H_{20}O_{10}$ is hydrolized and so on. According to this view there would always be combined maltose present in a starch transformation after a certain stage, till the hydrolization reached the point where only non-reducing dextrin is left (that is—taking the starch molecule as ten groups of $C_{12}H_{20}O_{10}$—when

only two such groups were left unhydrolized). The nearer a malto-dextrin group resembled in its structure and composition the original starch complex the more susceptible would it be to the action of cold-water malt extract (diastase) and vice versa. Enough has been said to show how varied the relationship of the products of starch transformations may be under varying conditions, not to speak of the variation in the character of the products themselves. We shall have something more to say about the practical outcome of the foregoing considerations under Brewing and Distilling, but a few words more about the substances as regards their behaviour towards yeast will not be out of place. They may be classified in this respect as follows:—

Starch transformation products.	Behaviour with ordinary brewer's yeast.
Maltose	Fermentable.
Easily degradable dextrins [1] . .	Non-fermentable as such.
Difficultly degradable dextrins [1] .	„ „
Easily degradable malto-dextrin [1] .	Slightly fermentable.
Difficultly degradable malto-dextrin [2]	Non-fermentable.
Non-reducing or end dextrin . .	„

Substances closely related to Starch.

Inulin $(C_6H_{10}O_5)n$ has been already mentioned in connexion with d fructose (levulose). It is a white powder which differs from starch in being soluble in warm water and giving no coloration with iodine. The sources have been already spoken of under levulose (d fructose). During the production of this last-named substance by the action of dilute acid or inulin, there are intermediate products formed just as in the case of starch and its conversion into glucose. An optically inactive disaccharide Synanthrose occurs associated with inulin in artichokes.

Glycogen $(C_6H_{10}O_6)n$ or *animal starch* is found in many animal tissues, but especially in the liver. It is also found in the vegetable kingdom, e.g. in truffles and in yeast. It can be obtained as a white powder soluble in water, is strongly dextro-rotary, and is coloured red by iodine. Acted on by diastase it yields maltose, and the action of dilute acid gives glucose. Glycogen forms a carbohydrate reserve in living organisms, disappearing with a state of hunger or deprivation of nourishment.

[1] Readily fermentable by yeast in presence of diastase.

[2] Slowly fermentable by yeast in presence of diastase.

Amylan α and β. Substances discovered by C. O'Sullivan in barley, which contains 2 per cent. of the α substance and 0·3 per cent. of β amylan. Both are laevo-rotary and give glucose when heated with dilute acid. They are interesting on account of their close relationship to starch. They are not coloured by iodine. α amylan disappears during the malting of barley and only a little β amylan is left, or some body resembling it, but more soluble.

Dextrin.

$(C_6H_{10}O_5)n$, say $C_{12}H_{20}O_{10}$.

It will have been inferred from the preceding allusions to dextrin that the term is more general than special. If it applies to any one substance more than another it is to the 'end' or undegradable dextrin of a starch transformation, a substance which is producible in very variable amounts according to the temperature of the transformation or the temperature to which the diastase has been previously submitted. The higher the temperature the more dextrin is produced in a given time up to the point of temperature at which the diastatic enzyme is destroyed. It is very difficult, but not impossible, to obtain dextrin free from reducing power as produced from the transformation of starch.

British gum or artificial dextrin, formed by heating starch to 150° – 160° C., consists of a mixture of maltose (in small percentage), erythro-dextrins in large percentage, achro-dextrin and optically inactive substances. True dextrin is a gummy, uncrystallizable, non-diffusible substance, having a high dextro-rotary power, viz. 200 AD. It is insoluble in alcohol, which provides a means of separation from maltose. It is practically unfermentable. It is possible to obtain starch transformations at the lower temperatures in which the dextrin is wholly degradable to maltose. There seems to be good ground for believing that the presence of dextrin has a restrictive action on the fermentability of maltose, varying probably with the kind of dextrin present. In the case of malto-dextrins the maltose by its association with a colloid substance is in a difficultly diffusible state, and this is doubtless the reason for the non-fermentability: it affords evidence, moreover, that substances have to enter the yeast cell in order to be fermented.

CONSTANTS FOR THE CARBOHYDRATES.

Taking 3·95 as divisor for solid matter.

	CuO per 1 gram.	Angular value AD.	Angular value AJ.	Divisor for AD (1 per cent. solution, 200 mm. tube).	Divisor for AJ (1 per cent. solution, 200 mm. tube).
Dextrin . .	Nil	200·0	222·0	4·0	11·56
Soluble starch	Nil	200·0	222·0	4·0	11·56
Maltose . .	1·37	138·75	154·0	2·78	8·02

Taking 3·86 as divisor for solid matter.

Cane sugar .	Nil	66·5	73·8	1·33	3·84
Dextrose . .	2·31	52·8	58·6	1·056	3·05
Levulose . .	2·205	−95·6	−106·1	1·91	5·52
Invert sugar .	2·25	−21·4	− 23·8	0·43	1·24

Dextrin and maltose brought to 3·86 values.

Dextrin . .	Nil	195·4	216·9	3·91	11·3
Maltose . .	1·34	135·5	150·4	2·71	7·83

CHAPTER VII

NITROGENOUS SUBSTANCES AND THE NUTRITION OF YEAST

WE may now pass on to the consideration of a class of organic substances which, although differing in a very material way from the carbohydrates both in chemical and physical attributes, are often found in association with them, and which stand in quite as important a relationship to the yeast organism as the carbohydrates do. The essential chemical difference is that the substances, in addition to carbon, hydrogen, and oxygen, contain the element nitrogen and a small quantity of sulphur. [A small quantity of phosphorus is generally found associated with nitrogenous substances of the nature of albumen, and in the case of a few substances the amount is considerable; it is not certain, however, in the majority of cases that phosphorus is a necessary constituent.] We may speak broadly of the bodies to be taken into consideration as nitrogenous substances, and deal with them in two groups. (1) The albuminoids—albumen and its immediate derivatives; (2) the amides or amido-substances.

Formerly the term proteid (from *πρωτεῖον*, pre-eminence) was used to signify the complex nitrogenous matters of plants and animals, the idea being that the proteids constitute (as they undoubtedly do) the chief components of living cells; but now that considerable additions to our knowledge have been made, one is able to refer the substances more nearly to their proper chemical grouping.

Albumen and the Albuminoids.

Albumen occurs in considerable quantity in both plants and animals, but is most familiar to us in the shape of egg-albumen or egg-white, which in the uncooked condition is a soluble or semi-soluble substance, being probably in the state of albuminate, for it has a good deal of saline matter associated with it. Egg-albumen in some of its physical attributes bears some resemblance to gelatinized

starch, for, like it, it appears to be in true solution when largely diluted with distilled water, but being colloidal in its nature it fails to permeate a porous membrane like parchment, or a porous substance such as unglazed porcelain. Egg-albumen in the coagulated state—which ensues at a temperature of 160° F. and upwards—is in a truly insoluble condition and requires special means to bring it into solution, such as the action of the digestive liquids of the stomach. The combined action of pepsin and dilute acids on egg-white gives rise to soluble peptones which are able to diffuse through a porous membrane. We allude thus early to the transformation of insoluble into soluble and diffusible albumen because we have the yeast cell with its enveloping membrane in our mind, and it is necessary that the substances which are dealt with in its economy should be able to permeate the cell envelope. The molecular structure of albumen must be very complex: possibly even more so than starch, but we have not the data that are available in the case of the latter substance to assign even an approximate formula to it. The evidence that is available points to a composition of about $C_{72}H_{112}N_{18}O_{22}S$.

Analysis of albuminoid substances from various sources gives the following figures as percentages:—

	Extremes.	Average.
Carbon	52·7 to 54·5	53·6
Hydrogen	6·9 ,, 7·3	7·1
Nitrogen	15·4 ,, 16·5	15·9
Oxygen	20·9 ,, 23·5	22·2
Sulphur	0·8 ,, 1·6	1·2
		100·0

The substance termed gelatin, which occurs in such considerable quantities in the bones and cartilages of the animal body, is rather closely allied in its composition to albumen, but differs from it in that it cannot readily be brought into diffusible and assimilable forms suitable for the nourishment of living cells.

Some of the chief reactions by which albuminoid matters are characterized are the following:—

The albuminoids or proteids when heated—say in a glass tube closed at one end—evolve ammonia, which may be recognized by the smell and verified by the usual tests, e.g. turning red litmus-paper blue, &c.

If albumen be diffused in water, and some strong nitric acid added, a white precipitate is formed which turns yellow on heating the liquid.

On adding ammonia to the cooled solution the yellow precipitate becomes orange-coloured.

One of the most characteristic tests is the so-called biuret reaction :—A few drops of a weak solution of copper sulphate are added to the aqueous solution containing the albuminous substance, and then a few drops of a strong solution of sodium hydroxide (Na HO), a colour is developed according to the kind of albuminoid substance present as follows :—

Albumins give a violet colour.
Proteoses give a reddish violet.
Peptones give a rose red.

All proteids, according to Hlasiwetz and Habermann, when boiled with dilute sulphuric acid, or better dilute hydrochloric acid and a little stannous chloride ($Sn Cl_2$), yield exact quantities of aspartic acid, leucine, glutamic acid, tyrosine and ammonia. We shall speak of the nature of these substances later on; in the meantime their enumeration will give some idea of the complexity of the substance or substances from which they are derived. The yeast organism (unlike the bacteria associated with ordinary putrefaction which break up albumen rather readily with not unfamiliar results) has no power of converting egg-albumen, either in the coagulated or uncoagulated state, to its own uses as a nutrient medium. So far as our knowledge goes, yeast can only apply to its nourishment diffusible nitrogenous substances present in the solution with it : it therefore becomes a very important question, from the point of view of the fermentation industries, as to the modes by which assimilable nitrogenous bodies are derived from non-assimilable, and the relationship in which the different derivatives of ordinary albumen stand, as based upon their solubility in water and other media; and the effect of raising the temperature of the solutions so formed.

Classification of Albuminoids.

Class I. Albumins. Soluble in water. Some coagulable at 160° F.
Examples : Albumen of blood serum, soluble in nitric acid.
Egg-albumen, almost insoluble in nitric acid.
Plant-albumen, almost insoluble in nitric acid.

Class II. Albuminates, derived from albumen; obtained by the action of acids and alkalies on albumen. Also includes metallic compounds of the albuminoids. Mostly insoluble in water.

Class III. Globulins and glutens. Insoluble in cold water,

slightly soluble in hot water. Soluble in dilute acids and alkalies. Soluble in weak solutions of sodium chloride (1 per cent.) and neutral salts. Soluble in 70 per cent. alcohol. Mostly coagulable at 140° F. Non-diffusible.

Class IV. Albumoses or proteoses. Soluble substances not coagulated by heat. Mostly precipitated on saturating the solution with certain neutral salts, e. g. ammonium sulphate and magnesium or zinc sulphates. Non-diffusible, or difficultly so; precipitated by tannic acid.

Class V. Peptones. Easily soluble, non-coagulable, not precipitated by saturation like the albumoses, precipitated by nitric acid. Readily diffusible; precipitated by tannic acid.

Class VI. Insoluble proteids. Includes coagulated albumens and a number of substances varying in their reactions. Some are acted on by pepsin yielding soluble syntonin and peptones.

It will be understood that the classification is rather general than special, for the properties of the albuminoids are so varied and the peculiarities of one substance so often differ only in degree from those of another, that sharp distinctions as regards characteristics are rather the exception than the rule.

Many chemical reagents have been spoken about at different times as group reagents for the albuminoids, but they have usually proved disappointing in their operation as they generally yield mixed precipitates instead of precipitating, as was supposed, only the albumoses or the peptones, as the case might be. The present state of our knowledge, therefore, does not permit of any rigid classification of the albuminoids, but it must not be thought that the attention given to these questions by various investigators has been ill-bestowed; on the contrary, it has indicated the lines on which further important results might be obtained.

For purposes connected with the growth of the yeast organism, and various phases of the fermentation industries, we may take it that the classification of the albuminoids is based on the following considerations:—

1. Whether the albuminoid substance is soluble in cold water?
2. If not in cold water, is it soluble in hot water?
3. If soluble, is it coagulable at a temperature of 140° F., or at higher temperatures—160° F. and upwards?
4. Is it thrown out of solution by saturating with a neutral salt?

5. If insoluble in water, is it soluble in feebly acid and alkaline solutions (0·1 per cent. hydrochloric acid), (0·1 per cent. caustic soda or caustic potash)?
6. If not soluble in above, is it soluble in stronger acid and alkali?
7. Is it soluble in 70–75 per cent. alcohol?
8. Is it precipitated by tannic acid or gallo-tannins?
9. Does its solution in conjunction with fermentable carbohydrates give rise to yeast crop on sowing with a small quantity of yeast?

A consideration of the albuminoids in connexion with the fermentation industries necessarily involves some attention being paid to their nature and occurrence in the materials used for the production of worts. In the case of grape juice or 'must' although there is no very precise knowledge of the kind of albuminoid substances present, analysis demonstrates the fact that they are present, and in quantity sufficient to afford the requisite nitrogenous aliment to the yeast and conduce to a vigorous fermentation attended by yeast crop, which could not, in the case of grape juice, possibly take place without this form of nourishment, seeing that fermentation is inaugurated by the comparatively small quantity of yeast derived from the surface of the grapes, and that it could not increase to the extent it does without proper aliment.

In the case of distillers' and brewers' worts our attention is claimed by the cereals used in their production, especially barley as barley-malt. A great deal of scientific investigation has been directed towards identifying the chief proteid or albuminoid constituents of the chief cereals, wheat, barley, maize, &c., but it would not be profitable to enter into much detail concerning them. For practical purposes we may take cognizance of the following facts. The cereals contain their albuminoid matter chiefly in three forms—

1. Readily soluble, but largely coagulable by heat.
2. Difficultly soluble (soluble perhaps to a certain extent in hot water).
3. Insoluble.

It is plain from the foregoing that an aqueous extract of the cereals which has been submitted to heat will contain but little nitrogenous matter, and this is indeed the case; and even if the solution were not heated, the albuminous matter present in solution would not be of a kind to afford any very perceptible nourishment to the yeast

organism. The question immediately arises as to what influence must be brought to bear upon the natural forms of albumen to convert them into yeast-feeding substances. One such process we have already alluded to, viz. the conversion of coagulated egg-white into peptones. We will describe this change a little more fully, as it is a very important one.

If ordinary egg-albumen be diffused in distilled water, and the mixture or solution is gradually brought to a temperature of 160° F. and upwards, on a water bath, the albumen separates in curdy flocks; let the liquid be now cooled to 100° F. and to about 500 c.c. volume, add one gram of pepsin and one c.c. of hydrochloric acid. Keep the whole at about 100° F. for some hours. If the pepsin be an active preparation (which is by no means always the case), the albumen will for the most part dissolve. If a portion of the solution be filtered clear and neutralized with, say sodium carbonate, practically the whole of the albumen is precipitated. This is spoken of as the acid-albumen stage. Let the bulk of the original solution go on digesting at 100° F. for some days: a portion then withdrawn yields practically no precipitate on neutralizing, but on saturating with a neutral salt, such as ammonium sulphate, almost the whole of the nitrogenous matter in solution is thrown down. This is described as the albumose stage. If the original solution be further digested at 100° F. for a few weeks, and no putrefactive decomposition has set in, a stage is reached at which neither boiling, neutralizing, nor saturating with a neutral salt, causes any precipitation. We have reached the stage of soluble and diffusible peptones, bodies which can nourish the yeast organism and other living cells. It need scarcely be said that the changes we have described, as going on slowly in solution, can be hastened by employing more pepsin, and it will be understood that they proceed with no little rapidity in the digestive economy of men and animals, whilst parallel changes proceed in the tissues of vegetable organisms.

In unmalted cereals very little of the soluble albumen is present in the peptone state, but unquestionably the processes set in train by germination cause a considerable amount of the albuminoid matter to be converted into such forms as will diffuse through the cell membranes of the cereal seed, and so nourish the different parts of the organism. Malted barley contains at least twice as much soluble nitrogenous matter as unmalted; and, although a good deal of it is coagulable by heat, there remains a considerable margin which passes

on into malt worts, and serves as nourishment for the yeast which is added to promote fermentation. Direct experiment has shown how well adapted to the nourishment of yeast are the peptones derived from the action of pepsin on the albuminoid constituents of fleshy substances and of egg-white; and, seeing how closely allied the nitrogenous components of plants are to those of animals, it is a fair argument from analogy that the vegetable substances corresponding to the meat peptones should have a marked nutrient power on vegetable organisms.

We will now try to summarize the more reliable results obtained by different investigators of the albuminoids of the chief cereals. Ritthausen, Kreusler, Osborne and Chittenden, appear to be the chief authorities.

THE ALBUMINOIDS OF BARLEY (Ritthausen, Kreusler).

Albumin		Extractible by cold water. Coagulates on boiling, after which it is insoluble in acids and alkalies.
Mucedin (Ritthausen, Kreusler) [probably same as Osborne's hordein]		Feebly soluble in cold water, somewhat more so in hot. Not easily distinguishable from gliadin or plant gelatin. Soluble in 75 per cent. alcohol.
Gluten-casein (Ritthausen, Kreusler)	[probably same as Osborne's glutenin]	Insoluble in cold water or hot. Closely associated with the phosphates of the alkaline earths. Soluble in 75 per cent. alcohol.
Gluten-fibrin (Ritthausen, Kreusler)		Insoluble in cold or hot water. Soluble in many dilute acids precipitated by alkali from these solutions. Soluble in 75 per cent. alcohol.

The following figures give perhaps some approximation to the albuminoids present in barley. They are Osborne's results:—

Albumin (leucosin)	0·30 per cent.
Albumoses or proteoses and globulin (edestin)	1·95 „
Hordein	4·00 „
Insoluble nitrogenous matter calculated as albuminoid matter	4·50 „
	10·75

In good malting barleys about one-sixth of this total would be soluble in warm water. In malt, nearer one-half the albuminoids are in a soluble state, having, as we have previously stated, undergone much modification. The additional soluble substances in malt are most probably albumoses and peptones, and the increase in the amount of soluble albuminoid matter is due to the action of certain substances named enzymes which are produced by the vital activity of the germinating corn, and are able to attack insoluble albuminous matter and convert it into soluble, very much as pepsin does. More exact nomenclature describes these active substances as proteolytic enzymes[1], and their formation is probably initiated by the action of the living cells of the germ upon soluble nitrogenous matter. Osborne and Campbell have found as the result of a careful chemical examination of malt, leucosin, globulins (edestin and derivatives) and proteoses. The albuminoids soluble in cold water constituted nearly 25 per cent. of the nitrogenous matters, and of this portion over 80 per cent. was coagulated by heat: their figures, however, show a total of nitrogenous matters a good deal too low for average English malts, viz. 7·84 per cent. of proteids instead of about 12 per cent.

Wheat contains proteids closely related to those in barley, but in addition a substance named gliadin (Ritthausen) is found in it which belongs to the gluten group, and, in association with gluten-casein and gluten-fibrin, furnishes the tough plastic mass which remains on kneading wheat-flour in a stream of running water. Gliadin is presumably absent from the flour of the cereals which leave no such plastic mass when treated in the same manner. Gliadin is soluble in dilute alkaline liquids, and is soluble in 75 per cent. alcohol.

Maize contains albumin, globulins (according to Osborne three), and a member of the gluten group which Osborne terms maize fibrin or zein, soluble in 75 per cent. alcohol.

The Amido-Substances.

We may now proceed to the consideration of another group of nitrogenous substances present in the cereals, viz. the amido-bodies—derivates of the amines—which are regarded by some as being the final decomposition products of the albuminoids, and derived from the

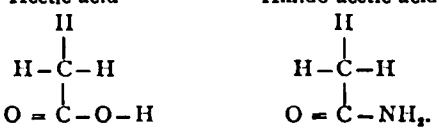
[1] A substance of this nature has been recently identified by Windisch and Schellhorn in germinating barley and malt. It is capable of liquefying gelatin.

peptones. Although the amides are complex in their structure, their chemical characteristics are decidedly more definite than those of the albuminoids, and the composition of many members of the group has been accurately determined.

Amines are chemically defined as bodies derived from ammonia by the displacement of its hydrogen wholly or in part by alkyl radicals[1]. If an atom of hydrogen only is replaced, then a primary amine or amido-base is produced, characterized by the amidogen group NH_2. Such substances can in turn form compounds with acids, and when the NH_2 group is attached to carbon in an acid, amido-acids are formed, the amidogen behaving as it does in amides or amines. Examples of amines:—

methylamine CH_3, NH_2 found in herring-brine.
ethylamine C_2H_5, NH_2.

Example of an amido-acid derived from acetic acid:—



The amides are of a crystalline nature and readily diffusible, and though they may be produced by the breaking down of albuminous matter, it is highly probable that they can also in the plant economy go to build up albuminoid matter.

The amido-substances which are of more particular interest in connexion with our main subject are:

Urea, $CO(NH_2)2$, the amide of carbonic anhydride, a product of the destruction of organic nitrogenous matters, amides and albuminoids, is found in animal juices. It is present in the yeast cell in small quantities. It is a white crystalline substance soluble in water and alcohol: it has a taste like saltpetre.

Asparagin, or amido-succinamic acid, originally obtained from asparagus juice and found in seeds during germination, is a substance which is capable of forming large colourless crystals readily soluble in water, but insoluble in alcohol. Two kinds of asparagin are known,

[1] The term alkyl or *alcoholic* radical is applied to the residual group of atoms derived from a paraffin hydrocarbon by the loss of one atom of hydrogen, thus CH_4 gives CH_3 and C_2H_6 gives C_2H_5.

one dextro- and the other laevo-rotary. The empirical formula is $C_4H_8N_2O_3 + H_2O$, and this extended becomes

$$\begin{matrix} CH(NH_2)COOH \\ | \\ CH_2CONH_2 \end{matrix} \quad \text{or} \quad \begin{Bmatrix} CONH_2 \\ CHNH_2 \\ CH_2 \\ COOH \end{Bmatrix}$$

Added in small quantities to solutions of fermentable sugars, asparagin forms an excellent yeast food, promoting a considerable growth of new cells, if other conditions are favourable thereto.

Aspartic acid, or amido-succinic acid, bears the following relationship to succinic acid:—

Succinic acid	Aspartic acid
$\begin{Bmatrix} COOH \\ CH_2 \\ CH_2 \\ COOH \end{Bmatrix}$	$\begin{Bmatrix} COOH \\ CHNH_2 \\ CH_2 \\ COOH \end{Bmatrix}$

A crystalline laevo-rotary substance which may be obtained by boiling asparagin with alkalis or acids.

Glutamine. A crystalline substance, having the composition:—

		Related to glutaminic acid	And to glutamic acid
$C_5H_{10}N_2O_3 =$	$\begin{Bmatrix} CONH_2 \\ CHNH_2 \\ CHCH_3 \\ COOH \end{Bmatrix}$	$\begin{Bmatrix} COOH \\ CH(NH_2) \\ CH_2 \\ CH_2 \\ COOH \end{Bmatrix}$	$\begin{Bmatrix} COOH \\ CH(NH_2) \\ CH_2 \\ CH_2 \\ CO(NH_2) \end{Bmatrix}$

It is readily decomposable by the action of alkalies and acids into glutamic acid and ammonia, and it is probably owing to this fact that glutamine is seldom met with in plants, whereas the presence of glutamic acid is frequent. Glutaric acid $\begin{Bmatrix} COOH \\ CH_2 \\ CH_2 \\ CH_2 \\ COOH \end{Bmatrix}$ homologous with malic acid, is another product of the action of hydrolizing agents, dilute acids, &c., on albuminoids.

Leucine, or amido-caproic acid, $CH_3.(CH_2)3\,CH(NH_2).COOH$, is a beautifully crystalline substance, obtainable in silvery white laminae: it is found in many animal juices, and is present in the yeast cell, being a product of the breaking down of albuminoids. It is slightly soluble in water, and is laevo-rotary.

Tyrosine. Hydroxyphenylamidopropionic acid.

$$\begin{matrix} C_6H_4 \\ OH \end{matrix}\!\!\rangle\!\!\rangle CH(NH_2)COOH.$$

Crystallizes in fine silky needles, soluble in cold water: the ordinary form is laevo-rotary, but a dextro-rotary form is known. Tyrosine is a product of the decomposition of albuminoids, and is present in yeast.

Several of the substances enumerated, although produced by the vital processes going on in the yeast cell, have been found to possess some nutrient power for the cell. (It is even affirmed that glycerin, which belongs to the true excreta of the yeast cell, has some nourishing power when fed to the cells in a saccharine solution.)

Probably all the amido-substances mentioned occur in malt wort and the beer produced from it, being derived either from the malt or the yeast added. A few other substances of this class, of rarer occurrence, have been also discovered in yeast and in beer.

The Enzymes.

Formerly spoken of as the unorganized ferments—a not altogether fortunate appellation—are a class of nitrogenous substances of the nature of albuminoids which are not directly associated with the nutrition of the yeast organism, but which play a very important part in the fermentation industries—brewing and distilling. For the purposes of the industries just mentioned, the chief enzyme is diastase, as it is commonly called, the active principle of malt, whose action on gelatinized starch we have already dealt with at some length. Unmalted cereals contain a form of diastase, and, in the case of barley, a considerable quantity of a soluble and coagulable albuminoid substance is present which, although unable to transform gelatinized starch, is capable of acting readily on soluble starch forming maltose and possibly dextrins. In other words, the barley diastase possesses, as regards starch, *saccharifying* but not *liquefying* power: it cannot make the first step of the reaction, viz. from gelatinized starch to soluble starch. Malt, on the contrary, has this power to a full extent, a power developed during the process of germination, and it may be said that the chief object of the malting process is to develop this particular phase of activity which, in conjunction with the saccharifying diastase, is needed to convert the remaining starch of the malted cereal into soluble carbohydrates by means of the mashing process.

The diastase of both barley and malt is soluble in cold water, and is most probably an albuminoid, or mixture of albuminoids, and like other substances possessing kindred properties, is most probably in some peculiar state of molecular equilibrium which is rather easily upset. The particular kind of energy that the enzymes exhibit has been made the subject of much scientific research, and the relationship in which they stand to the substances that they are capable of transforming or decomposing has been aptly compared by Emil Fischer to that of a lock and key. The action of the enzymes seems to be definite as regards the products obtained under like conditions, and conforms to certain laws which regulate chemical change. In the case of malt diastase the matter is complicated somewhat by the active agent having apparently more than one phase in its mode of operation; for instance, the action of invertase on cane sugar in solution is comparatively easy to follow, for it merely produces invert sugar or equal parts of glucose and fructose, but the action of malt diastase, or starch paste, is complex both as regards the quantity and quality of the products. On long storage of malt, the diastatic power, as it is termed, ebbs slowly away, rather like the vitality of a stored seed, and so is unlike the stored chemical energy of stable chemical substances like potassium chlorate, &c. The enzymic activity seems to be called into existence by the direct influence of living cells on albuminoid substances brought into the sphere of influence, and the power conferred like that from which it proceeds is, as it were, the *temporary* possession of the substances exhibiting it.

Two enzymes, cytase and peptase, will be alluded to under Malting: they have no direct connexion with fermentation.

Two other enzymes call for some further notice here. The first of these is the substance invertase, or invertin, contained in quantity in ordinary yeasts. Its action in producing invert sugar is a hydrolytic one, being expressed thus:—

		Invert Sugar		
Cane Sugar	=	Glucose		Fructose
$C_{12}H_{22}O_{11} + OH_2$	=	$C_6H_{12}O_6$	+	$C_6H_{12}O_6$.

The Saccharomycetes do not ferment cane sugar directly, but act on it first by means of the invertase they contain, the invertase itself remaining unchanged. This action almost unquestionably goes on inside the yeast cell, for it has been found by James O'Sullivan that living yeast cells do not yield up their invertase to water in which

they are infused. Invertase has been shown by Cornelius O'Sullivan and F. Tompson to be one member of a series of compounds which they term the invertan series. These compounds contain an albuminoid substance in association with a carbohydrate, and only one member of the series possesses the particular kind of activity we have been speaking of. C. O'Sullivan concludes that the invertase itself is not injured or destroyed by the act of inversion, and as a corollary of this it follows that there is no limit to its inverting power, the action, however—and this holds good for diastase and other enzymes —may become exceedingly slow owing to the presence of a high percentage of the products of the action in any given solution. J. O'Sullivan shows that invertase conforms to one of the chief laws governing chemical change—the law of mass, viz. if in a given solution 10 per cent. of the substance (e. g. cane sugar) which it contains is acted on in a given time, then in the next similar period of time 10 per cent. of the remainder will be decomposed and so on; in other words the decrease in the quantity of cane sugar hydrolized in a given time by invertase is proportionate to the decrease in the amount of unchanged cane sugar.

The fact should not be lost sight of that the presence of invertase has been found to be not uncommon in different parts of plants, e. g. the Gramineae, and it doubtless plays a very important rôle in plant economy by bringing the cane sugar into a state in which it is more directly suited to the nutrition of the plant cells; it is probable, moreover, that some of it goes to actually build up cell tissue. Enzymes are found in all living organisms, animal and vegetable, and undoubtedly perform functions of the very first importance.

Another member of the enzyme group to which we will devote a few words is the substance called glucase, present in yeast and in some of the cereals. This body is capable of producing glucose from the products of the action of diastase on starch paste, and thus of producing glucose from maltose. It was formerly believed that ordinary yeasts fermented maltose direct, that is, without previous hydrolization to simpler sugars; but it is now known that maltose, prior to undergoing fermentation, is converted into glucose thus:—

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + OH_2 = 2\,\underset{\text{Glucose}}{C_6H_{12}O_6}.$$

The substance zymase—already alluded to—which Buchner discovered some few years ago, and which has excited an extraordinary

amount of interest, may perhaps be regarded as belonging to the class of enzymes, although its properties are of a more complex nature. The recent investigations of Allan Macfadyen, Morris, and Rowland, on the expressed juice of yeast show that it can, like liquid yeast, enter into what is called auto- or self-fermentation, and that this process may take place in the cell juice to such an extent that the quantity of alcohol and carbonic anhydride produced, sometimes exceeds that yielded by a sugar solution treated with a similar quantity of the cell juice. Sugar has even been found in some cases to check this peculiarity of auto-fermentation. The most recent view expressed in relation to this extraordinary power of fermentative activity in expressed cell juice seems rather to favour the idea that the constituents of the cell juice retain vital activity for some time after being expressed from the cell, and that the action is due to a continuance of the function or functions. The action would seem to depend upon the formation of a compound of sugar with some substance—possibly an albuminoid—this compound breaking up into alcohol, carbonic anhydride and the original albuminoid or nitrogenous substance. This decomposition may take place either when the cell juice is added to a sugar solution, or in auto-fermentation owing to the decomposition of the pre-formed compound of sugar aforementioned. We may be pardoned perhaps for devoting so much space to this topic; the excuse lies in the deep interest and scientific import attaching to the matter.

Summarizing some of the chief points as regards the albuminoids, &c., we have:—

1. The ready solubility of certain albuminoids.
   The readiness with which they yield insoluble substances, as for instance by changes of physical conditions such as temperature and the action of chemical substances. Examples. Coagulation by heat. Action of acids.
2. The difficultly soluble nature of other albuminoid substances.
3. Solubility under special conditions, such as the presence of saline substances in solution, dilute acids and alkalies, alcohol.
4. The formation of soluble from insoluble nitrogenous compounds as for instance during the germination of seeds. (The malting process.)
5. The formation of substances assimilable by yeast and other living cells, involving the conversion of non-diffusible into diffusible substances.

6. Albuminoids, &c., of little solubility giving turbidity of solution with a slight change of physical conditions, such as temperature. (This is a fruitful source of trouble to the brewer in connexion with the clearness of his ales.)
7. The relationship between the albuminoids and the amides: the occurrence of both classes in cereals, cereal malts, and malt worts.
8. The enzymes. Soluble and usually coagulable albuminoids possessing a definite property or function which—broadly speaking—is that of converting insoluble bodies into soluble and diffusible ones.

Further Considerations about the Nutrition of the Yeast Cell.

We have already dealt with this matter in a very general way in one of the earlier chapters, but it is a subject which deserves a closer inquiry, and now that we have a clearer idea of the chemical nature of the substances generally, which play a part in connexion with fermentation, we are in a better position to consider their special relationship to the same.

A convenient way of dealing with the subject is as follows :—

1. Considerations respecting the constituents of yeast.
2. The alimentary matter—organic and inorganic—derived from :—
   (1) Natural fermentable liquids, e. g. fruit juices.
   (2) Artificial fermentable liquids, such as brewers' and distillers' worts.
3. The relationship between the ascertained facts of division 1 and those of division 2.

We have seen that yeast is of a vegetable nature and that its main constituents are water, carbohydrates, fat, nitrogenous compounds—albuminoid and amide—and mineral substances, and that its nutrition proceeds on the same lines as other lowly-organized plants. The soluble, diffusible nitrogenous compounds, which we have lately spoken of, viz. the peptones and amides, are referable to the nitrogenous side of yeast alimentation, whilst the mineral portion consists mainly of soluble phosphates or phosphates held in solution under favouring conditions. The fermentable sugars may, for our present purpose, be regarded rather as heat producers than as contributing to the building up of cell substance: their indirect effect, however,

in the particular direction of promoting growth of the cells, can hardly be dispensed with.

The best full analysis extant of the organic substances in yeast is that by Nägeli and Löwe, viz.—

		Per cent.	
Cellulose			37
Proteids	Ordinary albumen	36	
	Easily decomposable proteids similar to gluten casein	9	
		—	45
Peptone			2
Fat			5
Ash			7
Extractive matters			4
			100

This grouping will serve to give a general idea of the nature of the food required to satisfy the needs of the yeast cell as regards organic substances.

The inorganic constituents, or ash, figure thus :—

ANALYSIS OF YEAST ASH (average of three analyses according to Lintner).

	Per cent.	
Silica	1·34	
Iron (Fe_2O_3)	0·50	
Lime (CaO)	5·47	Chief compounds present.
Sulphuric anhyd. (SO_3)	0·56	Magnesium phosphate.
Magnesia (MgO)	6·12	Calcium phosphate.
Phosphoric anhyd. (P_2O_5)	50·60	Potassium phosphate.
Potash (K_2O) and a little soda	33·49	
	98·08	

The composition of the ash of brewers', or distillers' yeast (generally about 7·5 per cent. of the dry yeast) varies, as regards its components very much according to the amount of mineral matter in the waters used in the production of the worts as the following figures obtained by Matthews and Lott plainly show :—

	A. Yeast (from hard Burton water).	B. Yeast (from soft water).
Potassium phosphate	63·1 per cent.	93·9 per cent.
Magnesium phosphate	21·0 ,,	5·6 ,,
Calcium phosphate	13·6 ,,	traces
Difference = Silica, alumina, &c.	2·3 ,,	0·5 ,,
	100·0	100·0

The mean of these figures may be taken as representing sufficiently nearly the quantity in which the chief mineral constituents occur on the average, viz.—

Potassium phosphate . .	78·5	per cent.
Magnesium phosphate . .	13·3	„
Calcium phosphate . .	6·8	„
Silica, alumina, &c. . .	1·4	„
	100·0	„

Of these substances potassium phosphate must be regarded as absolutely indispensable, and magnesium phosphate as necessary. It is probable that calcium phosphate can be dispensed with by the cell. The small amount of sulphur in yeast is probably necessary to the cell economy. A. L. Stern has rather recently shown that sulphates are a very desirable if not necessary adjunct to the mineral nourishment of yeast. It is very plain then that so far as the mineral requirements of yeast are concerned, the liquids in which it is to normally develop must contain potassium, calcium, magnesium, phosphoric acid and some sulphates. As regards the kind of phosphate that may be desirable not much can at present be said, excepting that it would seem necessary that they should be orthophosphates or derivations of the acid H_3PO_4. The three bases enumerated form a great many compounds with this acid in which the hydrogen is wholly or partly replaced, so giving normal and acid salts. The acid phosphates of potassium—primary and secondary phosphates—are probably of more importance in connexion with cell life than the normal potassium phosphate. Potassium sulphate, produced by double decomposition between the calcium sulphate of Burton waters and the potassium salts derived from the malt, is unquestionably a good yeast nutriment, and tends to the maintenance of a definite type of alcoholic ferment such as the Burton yeast is. Grape juice, malt wort, and malt and cereal worts contain the materials from which yeast draws its supply of mineral matter, and in cases where, owing to the nature of the process employed and the type of yeast used as a ferment, mineral matter of some kind is lacking, a reinforcement of the supply of the particular substance is made possible by the character of the water supply used in the brewery or distillery; for whether hard or soft, it contributes some saline matter for mineral nutriment, though, of course, much more in the case of the hard water than the soft. Here we will leave this matter

for the time being and revert to the question of the kind of nitrogenous matter present in the yeast cell, constituting as it does near 50 per cent. of the weight of the yeast. Stützer gives the following figures, which must be taken as approximate:—

Per cent. of nitrogen present in the form of amides and peptones	10·1
" " " " albumin . . .	63·8
" " " " nuclein[1] . . .	26·1
	100·0

As knowledge advances the results of analyses of the organic components of yeast will doubtless be found very incomplete; we may, however, temporarily accept the figures as approximations, and discuss the constitution of yeast from the point of view they afford.

The simplest liquid in which yeast will grow (that is, multiply) and ferment is Pasteur's solution, which is composed thus:—

		Parts by weight.
Water	OH_2 . .	8,576
Cane sugar . . .	$C_{12}H_{22}O_{11}$.	1,500
Ammonium tartrate .	$C_4H_4(NH_4)_2O_6$.	100
Potassium phosphate .	KH_2PO_4 . .	20
Calcium phosphate . .	$Ca_3(PO_4)_2$.	2
Magnesium sulphate .	$MgSO_4$ . .	2

Now this liquid contains neither albuminoid nor proteid matter, cellulose nor fat, but it does contain the elements of these bodies arranged in a different manner. The yeast cells sown into such a solution can arrange these elements afresh, and form from them the complex substances that belong to its own economy. This power of constructing proteid matter out of a compound such as ammonium tartrate is distinctly a peculiarity of vegetable life, and tends to confirm the accepted idea of the vegetable nature of yeast.

Nägeli believed that the cellulose constituents of the cell wall, and possibly a portion of the protoplasm varying from 25 to 40 per cent. of the dry substance of the yeast, owes its origin to the sugar of the nutrient fermentable solution. It is certainly probable that there are normal carbohydrate constituents of the protoplasm, and it

[1] Nuclein, an albuminoid substance found in the nuclei of living cells, is distinguished from other proteids by containing a considerable percentage of phosphorus—about 9·6 per cent. Nuclein doubtless plays a very important part in the economy of living organisms.

has been suggested by Lintner that such is the case, and recent work has rendered it practically certain that the carbohydrate substance glycogen may be found in yeast cells at certain periods of their life history. This is a very interesting fact, especially as the glycogen appears to play a part similar to what it does in the animal economy, namely, a reserve material to be drawn upon in periods of rest or of deprivation of food. When yeast is removed from a healthy and vigorous fermentation it usually contains glycogen, and the cells colour reddish-brown with a solution of iodine of moderate strength; but during storage, and especially if the separated liquid yeast enter into the state of activity called auto-fermentation (during which alcohol and carbonic anhydride are produced), the stored glycogen wholly disappears, but may be renewed again by a fresh fermentation in a favourable medium.

The objection to yeast deriving any material amount of cell substance from the sugar fermented is that only about 1 per cent. of the sugar is unaccounted for by the products of its action. viz. alcohol, carbonic anhydride, &c. 100 c.c. of a 10 per cent. solution of cane sugar containing the necessary nitrogenous and mineral aliment is capable of yielding at the best some 5 grams of dry-pressed yeast (= 1·4 gram of dry matter)—say from 1 gram sown. The solid matter from the cane sugar unaccounted for by the products would be only some 0·1 gram. So that it is plain the sugar would have furnished very little of the 4 grams (=1·1 gram of dry matter) of actual new yeast-crop obtained, about 10 per cent. actually, which bears but a small relationship to the cellulose constituent, let alone the glycogen and other carbohydrates which may be present in the yeast cell.

There exists a great difference between yeast and another class of organisms—the bacteria—in regard to the albuminous matters it is able to deal with. The bacteria can readily attack raw or unmodified albumen, whereas yeast requires its nitrogenous nourishment to be in the form of soluble and diffusible peptones or amides, the products of the decomposition of albumen. Grape juice contains albuminoid matter in the required form, and so do also the fermentable fruit juices to a certain extent, and we have already spoken of the soluble albuminoid and amide substances present in malt and malt worts: all these liquids contain also the mineral nourishment which is essential to the well-being of the yeast.

Of the albuminoid substances proper there is little doubt that the peptones or kindred substances form the best nutrient matter for

yeast. Various amide compounds have been found to promote yeast growth, and of these asparagin has given the best results. The evidence obtainable rather points to the fact that amide nourishment is preferred by the yeast cell to peptone aliment. Very important results have followed experiments made with a view to ascertain what variations in the percentage of nitrogen are exhibited by yeast under diverse conditions of growth, and the effects traceable to such variations. Hayduck's investigations on this subject are of the first importance, and we will detail some of the conclusions arrived at by him and other experimenters.

The general tendency is for yeast to lose rather than gain nitrogen in a fermentation.

If the percentage of assimilable nitrogenous matter is small in relation to the saccharine matter the yeast loses nitrogen.

In fermenting plain solutions of sugar the loss of nitrogen is very considerable ; the yeast may just bud, but no new yeast is formed.

If the solution is rich in alimentary nitrogenous substances the yeast gains nitrogen.

The nitrogen may fall as low as 3·9 per cent. in the cell, or rise as high as 10·3 per cent. The percentage of nitrogen in the yeast accruing from any given fermentation also depends on the amount of new yeast formed. If little has been sown and the crop is considerable, the percentage of nitrogen will have most probably fallen from what it was in the sown cells. If much yeast has been sown with correspondingly little increase, the percentage of nitrogen will have risen.

Speaking generally, the fermentative power of yeast (measured by the products of alcohol and carbonic anhydride in a cane-sugar solution in a given time and under constant conditions) is directly proportional to the percentage of nitrogen it contains. But a yeast rich in nitrogen may prove a sluggish ferment in a saccharine liquid containing a large proportion of nitrogenous aliment; the yeast may have more tendency to produce new cells than to break up sugar, whereas had it been put in a plain sugar solution it would have fermented vigorously.

A yeast relatively poor in nitrogen may prove a more active ferment in a liquid containing a fair amount of nitrogenous aliment than a yeast richer in nitrogen, because it has less tendency to vegetate under the prevailing conditions ; in a plain sugar solution it would do less work than the highly nitrogenous yeast.

The nitrogen percentage of a yeast probably rises most in a liquid

in which the nitrogenous aliment is considerable and the cells non-multiplying.

Fermentation conducted at lower ranges of temperature—such as in lager-beer production—rather disposes yeast to take on a higher percentage of nitrogen. Fermentation of similar liquids or worts at a higher temperature diminishes the nitrogen in the yeast.

By aeration during yeast growth a decrease in the percentage of nitrogen is caused.

All these considerations are of great importance in their practical bearing upon the maintenance of yeast in a state of fermentative vigour, and they point to the desirability of, as far as possible, adjusting the quantity of yeast and the temperature of fermentation to the nitrogenous aliment that is at disposal in the liquids, worts, &c., to be fermented. It is necessary, for instance, that a brewer should keep his yeast in the most efficient state possible, and one of the chief factors in securing this is to brew with malt of good quality, for malt that is well made from good barley and in good condition contains within itself a sufficiency of nitrogenous aliment—if not of other kinds—to satisfy the needs of the yeast; but it is obvious that a considerable variation in the relationship of the various classes of nutrients, carbohydrate, nitrogenous, and mineral, may obtain according to the class of malt used.

From what has been previously said it will be gathered that there are means to hand for increasing or decreasing the percentage of nitrogen in any given yeast. If a yeast has become impoverished in nitrogen by a succession of fermentations in liquids lacking in nitrogenous aliment, liquids richer in assimilable nitrogen must be employed, and conversely saccharine liquids deficient in nitrogenous nutriment will bring down the nitrogen percentage. Between these two states there lies a median course whereby yeast may be retained at a satisfactory normal as regards reproductive power and fermentative power, and this desirable consummation has been reached in breweries that are able to continue using their own store yeast from month to month, and even year to year.

We have thus been again brought face to face with the double aspect of the behaviour of yeast in fermentation, the vegetative or reproductive aspect, and the purely fermentative one. This subject has been already dealt with at the close of Chapter IV, and we need hardly remark here that if we are concerning ourselves with the purely fermentative aspect of any given sample of yeast it becomes necessary

to perform the experiments under the conditions determined by Adrian Brown, viz. those of *non-multiplying* cells. If the reproductive side of the matter is the one under consideration, the quantity of yeast sown in a liquid must be adjusted so as to leave a good margin for increase.

All spontaneous or fortuitous fermentations of grape juice, &c., are cases where a small amount of yeast at the commencement of fermentation becomes a very considerable quantity towards the finish. Brewery fermentations are so contrived that the multiplication of the yeast is not inconsiderable—from three to five times the amount seeded in the worts. Distillers' fermentations may, as regards this particular feature, be divided into two groups. The Continental procedure is designed to obtain very large outcrops of yeast, say twelve times the quantity sown, whilst in England distillers' fermentations are more often of a non-multiplying character as regards the yeast; being sown or set with an excess of yeast in the first instance.

Summarizing, finally, the alimentary requirements of the yeast cell, we have three distinct classes—(1) the carbohydrate nourishment, (2) the nitrogenous, (3) the mineral. Neither of these groups alone will serve to promote both fermentation and yeast increase, and the carbohydrate nourishment is more a means to an end than a definite kind of food-stuff. It is the conjunction of the three forms that permits the yeast to normally develop: at the same time it should be remarked that there is still a good deal to be elucidated as regards the true relationship of the living yeast cell to the fermentable carbohydrates.

NOTE.—For some recent contributions to our knowledge of the matters relating to the nutrition of yeast, see Appendix (A. L. Stern's researches).

CHAPTER VIII

WINE—A SKETCH OF ITS PRODUCTION, THE MALADIES THAT IT IS SUBJECT TO, AND SOME NOTES ON FRUIT WINES GENERALLY

> From the sparkling fiery fountain
> And th' enchantment of the wine-cup
> Spring both poison and refreshment,
> What is mean, and what is lofty:
> Products of the drinker's fancy;
> Of his merit and endowments.
>
> Trans. from MIRZA SCHAFFY.

WINE, restricted in its meaning to the fermented juice of the grape—and no liquid from other fruits quite corresponds to the character and quality of the alcoholic beverage from this source—probably dates further back in its history than the cereal beverages; indeed, from the case with which a mass of crushed grapes, or the juice expressed from them, enters into fermentation, it may be concluded that the phenomenon of fermentation was speedily discerned whenever and wherever man's association with the grape vine commenced. Evidence respecting the occurrence of the vine greatly antedates our knowledge of the production of wine, for unmistakable fossil relics of the wild vine have been found in brown coal or lignite deposits at Salzhausen in Hesse-Darmstadt, and the shaft by which this layer of brown coal is reached passes through 180 feet of solid basaltic rock. Even the pips or stones are found, and some of the impressions of the vine-leaves are beautifully distinct. It is estimated that 100,000 years at least must have elapsed since the growth which indubitably took place on the spot where the remains have been found. To speak of the Egyptians, or the Greeks and Romans, as tenders of the vine and wine-makers is, after this, to deal with comparatively recent periods.

There is no doubt that accumulated knowledge of viticulture and

wine-production has been handed down traditionally, and through the Greeks and Romans it spread westwards till, at the present time, old methods have been greatly improved upon—more perhaps in connexion with the selection and training of the vines, than as regards points such as fermentation, storage, &c., which are included in the actual production of wine. Nevertheless, the whole now constitutes a distinctly scientific industry, and it covers so much ground, and embraces so much positive knowledge, that one would have to specialize either in the direction of the horticultural aspect of the matter, or the actual vinification processes commencing with the ripe grape, to do full justice to either. The soils that favour the growth of the vine are chalky, silicious, aluminous, and magnesian, granitic mountains, transitional strata, volcanic and alluvial land; also along the borders of rivers. A thorough knowledge of soils is necessary in order to produce the best results, and to decide as to the best dressings to apply to make up possible deficiencies.

It would be quite outside our province to say much about the culture of the vine: when it is mentioned that the varieties of the vine run into hundreds, and that every large wine-producing district has methods of viticulture peculiar to itself; that the grapes are harvested at different stages of ripeness according to the character of the wine produced in particular localities, and that there are innumerable points connected with soils, dressings, prunings, training and such-like, it will be recognized that a wide knowledge would be necessary to understand the requirements of one separate district, e. g. the Medoc, the Champagne, the Moselle, the Palatinate, &c. Suffice it to say that nearly all the cultivated vines have, in the first instance, grown in an indigenous semi-wild state, and have been brought to what they are by the accumulated care and skill of centuries. Formerly it was believed that the characteristic flavour of wines of different localities was due entirely to matters of soil, climate, and dressing; but recent investigations go to show that the more immediate cause is the particular yeasts whose cells are found on the grapes. In a few cases the special flavour of a wine proceeds from the grape—such as the Moselle flavour—but this is not the rule, for the 'must' or grape juice of one locality being fermented with Saccharomyces forms from the grapes of another, may have induced in it the characteristic flavour of the district from which the yeast is derived.

Vines can, no doubt, alter their characteristics in entirely fresh climates and on fresh soil, and give coarser wines—seldom finer ones;

but the subtler flavours of a wine—given a sweet clean 'must'—are due to the particular variety or varieties of Saccharomycetes.

For practical wine-making purposes grapes may be divided into two classes, white and blue-black, but between these are included yellow, yellow-green, bright red, violet, red, and blue. All these grapes, if they are quickly pressed after gathering, can be made to yield what are commonly called white wines, and, indeed, coloured grapes are not infrequently employed in this way. For a coloured wine a different procedure is employed, which results in the extraction of the colouring matter (oenocyanin) which is contained in the skins.

The juice of the grape is enclosed in thin-walled cellular tissue, and it is by the rupture of this, assisted by pressure, that the liquid known as 'must' is obtained. The chief components of this liquid are:—

Water;
Sugars—glucose and fructose, and some rarer sugars, e.g. inosit;
Acids—tartaric, racemic, citric, and malic;
Gummy substances, of the nature of pectin (soluble gum);
Albuminous matter;
Mineral constituents.

The sugar, as may be supposed, contributes the sweetness and provides the fermentable material.

The acids, in normal quantity, relieve the wine of what would otherwise be a rather mawkish taste; they have, moreover, a great influence on the quality and stability of the wine, and have much to do with the colour in the case of red wines, as the oenocyanin reacts something like litmus with acids and alkalies, and an acid reaction is necessary for a full bright colour.

The acids are partly free and partly in combination with bases (more especially with potash) in the form of tartrates, citrates, &c.

The chief function of the albuminoids is to provide nutrient matter for the yeast.

The gums and extractive matters give body.

ANALYSIS OF LIGHT 'MUSTS' FROM RIPE GRAPES.

	(1) A good year.	(2) A bad year.
Sugar	24·0 per cent.	18·0 per cent.
Acids	0·6 „	0·7 „
Water and extractive matters . .	75·4 „	81·3 „
	100·0	100·0

[The sugar in a 'must' averages about 20 per cent.]

(3) Analysis of a very heavy 'must' from aged grapes.

Specific gravity, 1,207·5 [water = 1,000].

Sugars	42·8	per cent.
Free acid	0·55	,,
Albuminoids	0·39	,,
Extractive matters, gum, &c.	14·03	,,
Mineral matter	0·76	,,
	58·53	,,
Water	41·47	,,
	100·00	,,

The ash of 'must' and wine contains the following substances (bases and acids):—

Potassium	Phosphoric acid
Sodium	Sulphuric acid
Calcium	Hydrochloric acid
Magnesium	Silicic acid
Iron (traces)	
Manganese (traces)	

[The average ash of wine is about 0·2 per cent.]

The more important compounds of the above entering into the constitution of the mineral matter are:—

Phosphates of potassium, calcium, and magnesium: especially the first-named substance.

Recalling to our remembrance the mineral requirements of the yeast cell it will be speedily recognized that they are adequately provided for in 'must'; all three classes of nutriment are represented in its composition—(1) fermentable sugars, (2) assimilable albuminoids, (3) mineral nutrients.

The skin of the grape, and not a little of the tissue of the fleshy part, consist mainly of cellulose. The skins are the repository of the colouring matter, and contain, in addition, tannin, and substances extractable during fermentation, which help to produce the aroma or bouquet of the wine. The colouring matter becomes soluble during the progress of fermentation owing to the influence of the alcohol produced, assisted by the fruit acids which largely come from the skin. A certain amount of tannin may come from the pips and a further quantity from stalky matter.

The relationship in quantity between the juice, skins, pips, stalks, &c., varies very much according to the kind of grape grown, and the degree of ripeness it is allowed to attain; and also as regards the

particular year or growth, and the age of the vine. In the earlier stages of the growth of the grape the sugar stands at a small percentage, whilst the free acid is at a large one; as ripening proceeds the sugar increases and the acid diminishes; for example: In a growth of Riesling grapes, when the sugar stood at 1 per cent. the free acid was 2·9 per cent., whilst seven weeks afterwards the sugar had risen to 18·4 per cent., and the free acid had fallen to 0·95 per cent. It can be gathered from this how necessary it is that the grapes should be ripe, and how adversely an enforced early harvest would affect the quality of the vintage. For vinification purposes there are practically three states in which the grape is gathered: (1) Ordinary ripeness, as for wines of ordinary or medium strength, e.g. average Rhine wines, medium clarets. (2) Full ripeness, as for the fullest clarets, and the finest Rhine wines. In such cases the grapes are not unfrequently left till they begin to wrinkle. (3) Distinctly overripe grapes, the juice of which has become much concentrated, and the wrinkling very apparent; this state is sometimes described as 'sweet rottenness,' and the heavy wines, the very full sherries and ports are made from grapes approximating to this condition. For such a wine as muscatel the grapes are sometimes brought more nearly into the condition of raisins by storing them after gathering, when they are spread out in thin layers and a considerable concentration of the juice takes place. A 'must' so obtained would correspond in composition fairly well with No. 3, p. 134.

The grapes being at the desired stage of ripeness—which more or less coincides with the state in which there is no further increase in the percentage of sugar, apart from the concentration of the juice resulting from over-ripeness—the vintage commences, and one of the most essential preliminaries is the thorough cleansing of all vessels which are required in the process. It is well if the grapes be gathered in fine weather, when the sun is up, not only that the dew may be dispersed, but that the grape itself may be somewhat warm, for this promotes a much more vigorous fermentation owing to the effect of temperature. In districts where the wines produced are of an average character, comparatively little pains are taken to separate sound from unsound or unripe grapes, but with finer wines great care is exercised. The general mode of procedure in the Rheingau and the Palatinate is as follows:—The grapes are gathered ripe, though perhaps with three different harvestings according to the kind of vine grown and the particular time it ripens. Mouldy, shrivelled, unripe, and spoilt

grapes are separated, also earth-smeared ones. The grapes are passed from the pickers' hand-baskets to large baskets or wooden skeps which strap on a man's shoulders, and as these are filled, the burden is discharged into a sort of tub on wheels, in which the grapes undergo a preliminary crushing by foot, being trampled as they are put in; the mass soon enters into fermentation. The tubs go to the press house. If a white wine is intended, the grapes are pressed before the incipient fermentation has made much progress, and the expressed 'must' passes to the fermenting vessel. In the case of red wines, the fermentation often proceeds to a far point in the wine press (the time the mass of semi-crushed fermenting grapes may stand varies from eight days to six weeks), and when the primary fermentation slackens pressure is applied to the partially fermented mass, and the separated wine is finished in suitable fermenting vessels; or the original mass of crushed grapes may be fermented in a special fermenting vessel, the wine drawn off by such an arrangement as that described further on, and the residual mass of skins then pressed.

The press house is usually contiguous to the fermenting cellar and the store cellar. The floor of the press house is smooth, being usually of cement or asphalt. Various patterns of presses are in use, ranging from the most primitive appliances to recently patented crushing and pressing apparatus. Considering the different way in which the grapes destined for the production of white wines are dealt with, as compared with red wines, it will be readily understood that the prolonged exposure of the fermenting liquid to the husks, pips, and stalks of the grape, results in bringing into solution a greatly increased quantity of tannin, tartrates, &c., &c., whilst the colouring matter itself is probably not flavourless, on the contrary, it is believed to contribute aroma as well as colour to the finished wine.

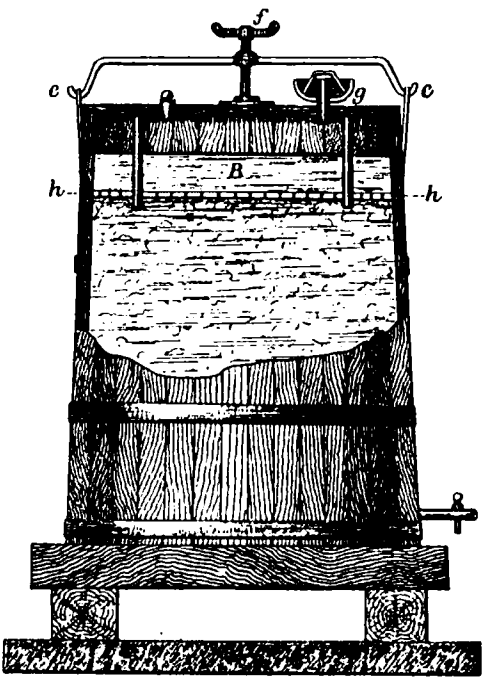
When the mass of crushed blue-black grapes is fermented, or if 'must' is fermented with the addition of some coloured grapes—as is sometimes done—the skins come in a body to the top of the liquid, forming what in France is called the 'chapeau.' In fermentations on a large scale this layer may be so dense and compacted that it will bear the weight of a man standing upon it.

The fermentation of white wines, and indeed of all wines, is preferably conducted in closed vessels, though perhaps it is not so absolutely necessary in the case of red wines, when the chapeau forms a protective coating, as also the layer of carbonic anhydride above the hapeau for which there should be sufficient head-room in the vessel.

A small quantity of fatty or oily matter comes from the stones, and this helps to seal up the top of the fermenting liquid from the influence of atmospheric air, for the great risk at this stage is acetification resulting from over-exposure, a change which will very quickly run up the acidity of the wine above the normal, and greatly deteriorate its quality.

Sketch of Fermenting Vessel.

This consists of a conical wooden vessel with a lid fitted in such a way as to be air-tight, and capable of resisting some degree of pressure. The charge of fermenting, crushed grapes being introduced, the strainer *hh* is brought down on the mass and keeps the skins continually immersed. Fermentation proceeds in the mass, and as carbonic acid is liberated pressure accumulates, but is prevented from becoming excessive by the valve *g*, or the gas can be liberated at will by removing the small plug in the cover. The main tap to the vessel is provided with a strainer to hold back the skins when the fermented wine is run off. Fermentation under these conditions can be conducted in a far more satisfactory manner, for the driving out of the air in the first instance by the carbonic acid, and the subsequent exclusion of oxygen, permits a wine to be produced singularly free



ee-Stirrup catches for fastening down lid.
f-Screw for tightening lid. g-Pressure Vent.
hh-Strainer & Supports
B-Clear liquid above the strainer.
(AFTER ANTON PIAZ)

FIG. 10.

from any acid produced by oxidation changes, especially from acetic acid, and also tends to inhibit the growth of mould and organisms of an undesirable character. The wine thus produced is said to be, moreover, more free from tannin derivatives than where the fermentation has been conducted with full exposure to the air.

The time occupied by the principal or primary fermentation is variable, and is practically over in from six to eight weeks: the time depends on climatic conditions, the character of the grapes, and other considerations, such as the temperature of the cellar, which varies from about 55° F. to 68° F., a very wide range. In a bad season there is less sugar to ferment and more acid to give harshness, whilst the contrary obtains in good years.

In a poor season the question often arises as to how the 'must' can be artificially strengthened. The Greeks and Romans used honey for this purpose: the modern substitute is sugar, and sometimes alcohol in the form of 'silent spirit,' or a low quality of brandy. When the acidity is high, the 'must' is sometimes diluted to normal acidity with water, and sugar is added to bring up the strength (Gall's method; German *gallisiren*). Another method introduced by Chaptal is to partially neutralize the acid with pulverized marble and then to add sugar. Another of the economies (if not adulterations?) of the wine industry is to exhaust the pressed skins, 'marc,' with a solution containing sugar and some alcohol; this extracts the remaining 'must' together with colouring matter, &c., and from the liquid a kind of wine can be made: this is known as l'etiot's process.

A certain amount of aëration of the 'must' is desirable prior to fermentation, and such admixture of air as results from the ordinary handling seems to suffice; but there are cases in which a direct rousing of the fermenting 'must' gives a healthy and needed stimulus to the yeast. Anything like a free circulation of air in the fermenting and storage cellars is deemed inadvisable, in order to guard against acetification.

The forms of Saccharomyces that mainly figure in wine fermentation are—

Saccharomyces Ellipsoideus, S. Apiculatus, and S. Reessii, of which the first-named is the prevalent one.

These ferments come, as we know, directly from the skins of the grapes, and although quiescent or dormant, they are very quickly brought into a state of activity when immersed in grape juice.

The secondary or cask fermentation is a very prolonged affair, and for its completion requires a period of some months. In Germany the slowly fermenting wine is drawn off or 'racked' two or three times from the lees into fresh vessels, and the oxygenation which then ensues gives a fresh stimulus to the fermentation, till after, perhaps, a third racking the fermentation comes to an end. The first transfer may take place in December or January; the second at the end of March or the beginning of April, and the third at the end of June. The wine is then stored in cask till such time as it is ready for bottling, when it is dealt with either on the spot, or at the place to which it is consigned. It is important that while the wine is in cask any shrinkage of volume should be made good periodically by filling up.

We may now institute a comparison between the composition of a young wine and the partially fermented 'must' from which it was derived:—

1. The unfermented sugar present in the 'must' ferments for the most part, and thereby the total of the alcohol, carbonic anhydride, glycerin and succinic acid in the wine is increased.
2. Owing to the increase of alcohol, a considerable separation of tartrates takes place, and in addition some of the colouring matter rendered insoluble most probably by oxidation changes.
3. Oxidation also leads to the precipitation of the greater part of the albuminoid matters.
4. New aromatic substances are formed.
5. In the case of white wines, some colouring matter is formed by the action of oxygen on the tannin and extractive matters.

The average composition of medium clarets and Rhenish wines may be thus represented:—

Alcohol	10·00	per cent.
Sugar	1·33	,,
Tartaric acid	0·40	,,
Acetic acid	0·10	,,
Tannin	0·15	,,
Succinic acid	0·12	,,
Albuminoids	0·10	,,
Glycerin	0·50	,,
Extractive matters	0·68	,,
Ash	0·25	,,
	13·63	,,
Water	86·37	,,
	100·00	

Other constituents not classified separately, present in small quantities:—

Malic acid	Aromatic substances
Racemic acid	Gum
Citric acid	Fatty matters
Aromatic ethers (chiefly oenanthic)	

The most important constituent of wine is, no doubt, the alcohol, for the quantity present determines the strength and very largely the value. Low-strength wines carry about 9 per cent., medium strength 11–12 per cent., strong and very strong wines up to 15 per cent.; a percentage of alcohol beyond this last figure is usually arrived at by adding alcohol (fortifying). Most of the original tartaric acid of the rough wine is thrown out during maturing as tartrate of potash containing some tartrate of lime: oxidation products of the tannin and colouring matter are precipitated at the same time, the whole constituting what is known in commerce as crude tartar; so that the net result is a lessening of the acidity (or, at all events, one phase of it) and a smoothing of the flavour, the bouquet increasing meanwhile.

White wines containing, as they do, so little colouring matter, tannin, and tartrates, are but little prone to throw deposits after the completion of the secondary fermentation. A small deposit noticeable in some white wines is often due to the use of sulphur or sulphur compounds (e.g. bisulphite of lime) applied either by burning sulphur in the casks or adding calcium bisulphite to the wine. The precipitated matter is in all probability albuminous in its nature.

The free acids tend to preserve a wine, as does also the relatively small amount of tannin present. Both tend to arrest or retard bacterial change, the first-named by a direct inhibitive influence, the last by the fact of its being in combination with the albuminous matter which might otherwise afford a ready food supply for bacteria. The alcohol should, however, be regarded as the main preservative in wine.

The flavouring matters of the wine, together with other substances which lend bouquet or aroma, are chiefly fragrant ethers of which the chief is oenanthic ether. The total quantity of ethers is very small, and large volumes of wine would have to be dealt with to furnish small visible specimens of them; the effect, however, which they produce is obviously very considerable. These ethers probably have their origin, for the most part, in certain chemical changes brought about in the alcohol, partly by the Saccharomycetes partly by incursive organisms, moulds, and bacteria, the quantity of ether

produced being determined to a not inconsiderable extent by the temperature at which fermentation takes place. The higher the temperature the more ethers are produced, but it does not follow that the bouquet will be improved, for the aroma of the additional ethers may not be of a desirable kind. Slower fermentations at the lower ranges of temperature give more satisfactory results than rapid ones at higher temperatures.

Sketch of the Vinification Process in the Burgundy District.

The grapes are packed into small baskets, transferred to larger ones, and then to an oval tub on wheels in which each portion of grapes is stamped upon and crushed in a very rough and ready fashion by a man standing in the vessel. The tub when filled is brought to the edge of a large vat, and the mass of crushed grapes is partly emptied and partly pulled out by a hooked implement from the oval tub. The mass then ferments for from four to six days. The skins rise and form a compact 'chapeau' which after a few days more sinks in; this state of things is often anticipated by a workman or two suitably unclothed getting upon the mass and breaking it in by stamping and plunging about on it. This is one of those primitive modes of procedure that causes the reflection that it is well not to inquire too closely into methods, but to enjoy without question. The temperature in the fermenting vessel or 'cuve' during vigorous fermentation ranges from 77° F. to 99° F. according to locality, &c. The primary fermentation being nearly over, the wine is siphoned off from a wicker enclosure which runs down the side of the 'cuve,' and which acts as a strainer: the wine so removed passes into another vessel to undergo secondary fermentation. Meanwhile the mass of skins and grapes which have escaped the preliminary crushing is crushed in a press, and thus a new quantity of sweet 'must' is obtained and added to the wine which is three-quarters fermented. A more powerful secondary fermentation is thus induced which is regarded, and perhaps justly, as being very advantageous. Sometimes the pressed mass or 'marc' is dealt with by Petiot's process. The wine drawn from the 'cuve,' plus that from the press, is gathered into casks of about fifty gallons' capacity, and the fermentation is completed in these in a suitable cellar, the wine being ready for sale in the March following the vintage; and when of ordinary quality it is fit for consumption at the end of the first year. Good wines require four years in cask and several years in bottle before they develop their final qualities.

Sketch of the Champagne Process.

Champagne may be looked upon as typical of a class of wines of comparatively limited production known as sparkling wines. These wines contain carbonic anhydride in such excess that it is liberated when the cork is withdrawn from the bottle. A comparatively small proportion—about one-fourth—of the grapes grown in the champagne districts is used for the production of sparkling wines, the remaining three-fourths are mainly used for the production of red wines, the mode of manufacture of which is nearly identical with that of Burgundy. The grapes destined for the production of champagne are very carefully picked in order that only sound ripe grapes may be used. 'The animation of a harvest day in the champagne district can hardly be imagined. Throughout the green undulating vineyards hundreds and thousands of people are dispersed; all the roads are lined with the cleaners and the heaps of grapes on trays and in panniers. Everywhere donkeys stand waiting for new loads or move in long strings along the narrow paths or the driving roads[1].' The grapes are brought into large and very powerful presses, and each pressing is completed in about two hours, otherwise colour would be obtained. [The mass remaining in the press is generally used for the production of red wine, the 'must' from white grapes being thrown upon the cake from coloured grapes, and allowed to ferment in contact with it in large vats.] The 'must' obtained from the first few pressings—but not the last—is run into large 'cuves' or vats, and these stand in a cool place for eighteen hours or so, during which period a scum is formed and certain matters are deposited: the 'must' now in a state of incipient fermentation is racked off from these impurities into casks of about the capacity of one hogshead. In these the now actively fermenting liquid cleanses itself by discharging yeast-dregs, &c., from the bung-hole, any space being made good by filling up periodically. The primary fermentation being well over, the wines are blended in order to secure certain standards of flavour and character and are racked into other casks. The wine is next fined with isinglass finings and then rests for a few weeks: at this stage the wine should contain 11 to 12 per cent. of alcohol, and about 2 per cent. of sugar should be still unfermented (the original sugar of the 'must' being about 20 per cent.). The wine is now bottled (*tirage*) and a slow fermenta-

[1] From *Origin, Nature, and Use of Wine*, Thudichum and Dupré.

tion in bottle goes on through the spring and summer, accompanied by a loss from bursting bottles ranging from 5 per cent. to 20 per cent., according to the season's vintage; rebottling is sometimes necessary to bring this secondary fermentation to a finish. The bottles are placed at such an angle in suitable racks that the sediment falls towards the cork, and is made eventually to settle upon it by an axial twist being given to the bottle from time to time by hand. This sediment is mainly yeast, and may vary in quantity from a barely perceptible layer to one nearly an inch thick. What is termed 'disgorging' now takes place. A skilled workman taking the bottle from the rack and keeping it in its relative position nearly extracts the cork, then turns the bottle neck upwards and liberates the cork; the escape of carbonic anhydride and the consequent foaming brings away the whole sediment. The neck is quickly cleansed and a temporary cork put in, and the bottle is passed on to workmen who by aid of a machine runs in a certain amount of cordial or liqueur (every firm has its own peculiar receipt), and corks and wires the bottle. Sometimes the disgorging has to be done twice over before the wine can be liqueured and finished. A highly sparkling wine has a pressure of from four and a half to five atmospheres: pressures of less than four atmospheres give a sparkling or creaming wine.

ALCOHOLIC STRENGTH OF WINES AND SPECIFIC GRAVITY OF THE ORIGINAL 'MUSTS' (WATER = 1000).

	Present spec. grav. of the wine.	Per cent. of alcohol weight on volume.	Calculated spec. grav. of 'must.'
Claret (good) . . .	995·0	8·6	1089·1
Carlowitz . . .	991·8	10·4	1090·2
Niersteiner . . .	995·6	7·6	1067·2
Steinberg Cabinet . .	992·8	10·2	1088·9
Marcobrunner . . .	994·8	10·5	1092·8
Sauterne	993·7	11·7	1105·4
Burgundy . . .	991·3	10·5	1089·4
Sillery	1010·9	10·4	1108·0
Manzanilla . . .	985·9	16·0	1134·5
Natural Sherry (so called)	1009·3	15·1	1128·5
Marsala	995·9	16·4	1147·7
Port (1851) . . .	999·2	17·5	1160·0
Natural Port (so called) .	997·1	13·8	1129·2

The materials for the foregoing table have been derived from

Thudichum and Dupré's work on wine, the original gravities having been calculated from the specific gravity of the distillates and the specific gravity of the spirit-free wines, and may be regarded as fairly close approximations to the actual original gravity of the 'musts.'

The Diseases or Maladies of Wine.

This subject was exhaustively dealt with by Pasteur in a spirit which was not only truly scientific but patriotic. The results of his investigations are detailed in his *Études sur le Vin* published in 1872. Some of the causes of deterioration of wine in cask and bottle lie rather deep, but in other cases the cause is more directly apparent. Piaz in his work on the preparation of wine[1] says:—'The best means of preventing the deterioration of wine are a careful and systematic method of production and cellarage; the most scrupulous cleanliness in all processes, apparatus, and vessels, and a sufficiently high percentage of alcohol in the finished wine.'

This excellent advice applies in a large measure to all fermented beverages. The chief defects to which wine is liable are the following:—

1. The formation of Mycoderma Vini as a film-growth following moderate exposure to the air, due to the incursion of the distinct organism which has been alluded to in an earlier chapter. The effect is to somewhat rapidly destroy alcohol, and to communicate an insipid or even strange flavour. The chief causes are most probably, flatness, low percentage of alcohol, high percentage of albuminoids and phosphates. Mycoderma Vini growing alone does not rapidly or hopelessly deteriorate a wine, if the growth does not go too far. This organism seldom forms when the amount of alcohol is over 12 per cent. The French name for this malady is *fleur du vin*. 'Ascescence' is the term used when a growth of Mycoderma Aceti accompanies Mycoderma Vini: the destruction of alcohol by the latter very much favours the appearance of the former.

2. Mycoderma Aceti: a direct cause of acetification by the production of acetic acid. The name may be taken as generic for the acetic ferments, of which there are several. They belong to the family of Schizomycetes or bacteria. The vinegary taste induced by a free growth of these organisms (the acetic ferments) is generally accompanied by a marked precipitation of colouring matter. The causes

[1] 'Die Weinbereitung,' Hartleben's *Chemisch-technische Bibliothek*.

are probably very much the same as those tending to a growth of Mycoderma Vini; both organisms require a certain amount of free oxygen to initiate the growth, and oxygen in some assimilable form for the continuance of it. The normal acetic acid of wines, does not exceed 0·2 per cent. An example of a deposit from wine which has suffered from Mycoderma Aceti and general bacterial change and deterioration, is shown in Plate VII.

3. Bitterness (Fr. *amertume, goût de vieux*), in some cases due to a definite organism—a bacterium showing chaplets of small bead-like forms (streptococcus)—but generally due to the mould organism known as Oidium Vini, and the bacterial organisms commonly associated with its growth. All red wines are subject to bitterness, especially those of the best 'crus.'

4. Oidium Vini (Plate VII): a mould growing submerged in the wine affected, the usual signs being a mousy smell resulting from the production of acetamide, and as there is often a concurrent growth of some acetifying organism, acidity sets in as well, the wine becoming undrinkable. Colouring matter is rapidly thrown down during the progress of this malady, which may affect even fine vintage wines (some of the 1875 clarets were spoilt by it). It is pretty certain that oidium infection comes direct from the vine, which has become subject to this mould owing to the weakening effect on the plant-growth, caused by the ravages of the phylloxera.

5. Viscous change (Fr. *graisse*): a curious malady which results in the wine becoming thready, oily, or so viscous that it can be drawn out like birdlime. It is not necessarily accompanied by acidity. The change is due to specific organisms, such as Pediococcus acidi lactici, and Bacillus viscosus. The disease is manifested in wines deficient in tannin and of low alcoholic percentage; the viscosity is most probably due to a decomposition of the remaining sugar into plant mucilage (viscose) and mannite.

6. Foulness: the development of an unpleasant smell in wine due to the formation of sulphuretted hydrogen and other sulphur compounds, probably mercaptans or sulphur alcohols. It may sometimes have its origin in the presence of free sulphur in the fermenting wine, due to a too liberal use of sulphur as an antiseptic in the vessels (the sulphur is burnt in them), or the use of bisulphite of lime, or even gypsum in excess. It is also believed to arise from an excess of albuminoid matter in the wine. It is interesting to note that beers are sometimes affected by this complaint, but it

is possible for both classes of beverage to entirely recover their good qualities. In the case of wine, other causes of the malady are the growth of the vines on sulphurous shales, the extensive sulphuring of the vines in combating phylloxera, &c., and the use of sulphites and bisulphites as antiseptics.

Summing up the chief causes of deterioration of wine we have:—

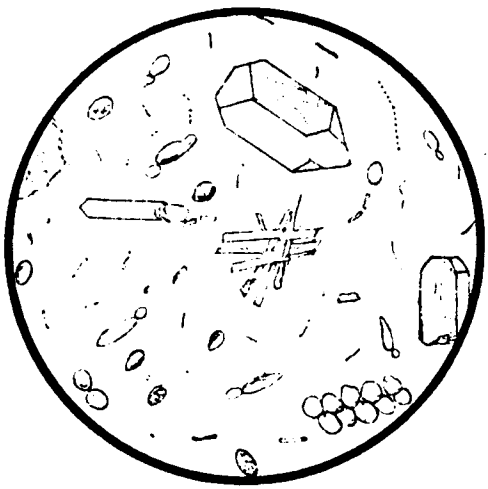
Deterioration of the vine itself due to improper cultivation, dressing, &c. Ravages of blight. Bad seasons for both growth and harvest, leading to high acidity in the 'must,' and small sugar percentage, which causes a low percentage of alcohol; the other constituents of the 'must' are probably in a more or less abnormal condition. Errors in manipulation. Uncleanliness. Over-exposure of wines. Too frequent rackings. Cellar temperatures too high.

It would be outside our scope to devote more than a few words to fruit wines from other sources than the grape, interesting though such manufactures may be, and palatable as such beverages not unfrequently are if carefully made. Indeed it is surprising what results can be achieved with, perhaps, gooseberries or rhubarb as the raw material, and how closely these wines may approach in character to unliqueured wines like Saumur. The fermentation of fruit juices generally may set in spontaneously on crushing and expressing the juice, or it may be assisted or regulated by the addition of yeast. The composition of grape 'must' may be taken as typical of fruit juices in general, divergences in the quantity of the several components being allowed for.

Cider and Perry.

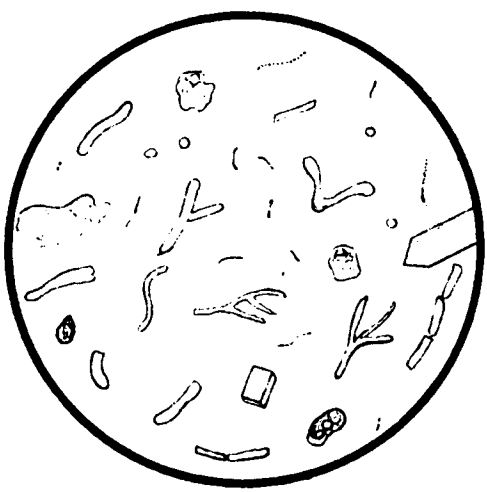
These beverages may be regarded as the wines of districts where the grape does not flourish or where no attention has been paid to its cultivation. The manufacture of cider is a by no means unimportant industry in certain localities both in this country and abroad. To obtain a cider of excellent quality it is not only necessary that a high-class apple should be employed, but that as much care should be bestowed on the process as if the intention were to make a first-class wine. The later-ripening apples, although somewhat harsh and hard, give a cider of great stability, and the kind of fruit should be so chosen as to procure a flavour which is more that of wine than of the particular apple employed. The fruit should be gathered in dry weather and a careful sorting out of any damaged or rotten apples be made. The methods of pressing vary not a little, but in general resemble those in use for the grape. Abroad, a kind of pug-mill with

PLATE VII.



Deposit—Sour Wine.

x 300.



Oidium Vini.

x 300.

stones revolving in a circular trough is often employed, as also patented apparatus of an improved character for cutting up the apples and pressing them: in either case it is not advisable to let the crushed or cut apples stand for long without getting the juice away from them, otherwise a high-coloured cider may result. On pressing, the expressed juice is run into casks with a fairly wide bunghole and fermented. In some of the manufactories conducted on modern principles, a selected yeast is added to secure a vigorous fermentation and a choice flavour—otherwise the fermentation is what is called a spontaneous one. The first juice expressed from the apples is the best, and may be worked separately from the later pressings. During the fermentation in cask the yeast rises and flows as a frothy scum from the bunghole, the space arising from the discharge being filled up from time to time, and this goes on till the primary fermentation is practically over. A patent vent is now inserted in the bunghole, which allows some little pressure to accumulate, and after about six weeks the cider is racked off by a pump into clean sulphured casks. It may be twice re-racked after this, and before the last withdrawal is fined with isinglass finings. The cider becomes smooth and bright, and retains a fine colour if it has not been unduly exposed to the air in the racking processes. Cider is normally somewhat weaker than wine and contains malic not tartaric acid. If a stronger cider is desired, sugar is added to the apple juice prior to fermentation. Abroad alcohol is sometimes added to the finished beverage. If the acidity of the original juice is very high it is sometimes reduced by Gall's process, i.e. dilution with a solution of cane sugar. When the cider is destined for bottling (champagne cider) the aroma or bouquet is sometimes enhanced by crushing some quinces with the apples.

Perry.

Pears are naturally richer in sugar than apples, and contain less proteids, fruit acids, and tannin; the resulting fermented beverage is therefore of a finer character, but is not nearly so stable. A bottling perry may be prepared in the following way:—The fruit is peeled and the cores and pips removed; then the fruit is broken up and pressed. The juice remains four hours in open tubs, when a deposit is formed and the liquid is then strained through linen in large funnels into the casks. A certain quantity of sugar is added, and a small quantity of tartaric acid. The casks are rolled over, and then a piece of linen is put over the bunghole. Fermentation sets in, and from this point the treatment is very much the same as it is in the case of cider.

CHAPTER IX

Introduction to the Malting and Brewing Processes and Description of the Malting Process

Introductory.

The rapid progress of knowledge and the increasing facilities for the communication of thoughts and ideas, have in recent years led to a keenness of competition which has had a truly remarkable effect upon the scientific industries. Brewing and, we may add, the spirit and yeast industries, have been amongst those which have profited largely by scientific investigation and have indeed been lifted by it quite out of the region of empiricism. It is not so long ago since it was said, 'any old woman can brew,' and in 'the good old times' there were, doubtless, plenty of housewives who could satisfy the not very critical beer-drinker of the day with the results of their efforts: nowadays it is more than probable that they would be invited to betake themselves to solitary and undisturbed enjoyment of the product of their skill. The moderately critical ale-drinker at the present time looks for characteristics which call for the fullest exercise of the brewer's art to produce them with regularity and uniformity in one brewing after another. We may mention the following:—A state of almost perfect brilliancy; a delicate aroma and flavour; a pronounced but not clinging bitter; a moderate percentage of alcohol, and the presence of enough carbonic acid gas to redeem the beverage from the imputation of being flat. British beer is manufactured mainly from malted barley, hops, and water; but a proportion of the malt is not unfrequently replaced by carefully prepared sugars[1] and skilfully made preparations of some cereals other than barley. The annual dictum of the Somerset House Laboratories effectually disposes of the not unfrequent and somewhat sweeping charges of adulteration brought against the cereal beverage

[1] This statement might be considered open to objection in face of the recent arsenic trouble, but it nevertheless is accurate as regards sugars in general.

of this country. For the brewing of sound and good ales, sound and good materials are required, and a most careful selection has to be made by keen judges from the various samples of barley and hops which are offered in any given season, the produce of one country being compared with that of other countries, so that the barleys which are best suited for malting, and the hops which combine the requisite strength and aromatic properties may be employed: thus it happens in some years that foreign barleys are preferred to home-grown ones, and a larger quantity of foreign hops may be used for the same reason. Science does not perhaps enter so largely into what may be termed the preliminaries of brewing, for the educated hand and eye—and we may add nose—will give a fairly adequate idea of the value of barley and hops: it is in the subsequent dealing with these materials that the higher science comes in. We have spoken of water as one of the materials. Good beer requires good water, and delicately flavoured pale ales can only be brewed with relatively pure waters of a certain composition. Chemical analysis steps in here and tells the brewer what constituents of water are desirable on account of their favourable action, and where the water may be found which contains them. This line of investigation has resulted from the fact that the wells in the valley gravels of Burton-on-Trent and the artesian tubes and bores driven into the Keuper marls underlying the gravels in the same locality, furnish a kind of mineral water which has, in conjunction with a high quality of malt and hops, produced the pale ales for which the metropolis of brewing has so long been famed. In other parts of the kingdom in which, owing to a similar geological conformation or from other causes, chemical analysis has shown the existence of similar water-supplies, beers comparable with those brewed in Burton have been produced. So much for the present as regards brewing waters, some of which are favourable to one system of brewing, some to another. The process of malting may be said to be governed by chemical, physical, and biological principles, for the germination of barley on the malting floors is a vital process, which sets in train a whole series of chemical changes—controllable in a certain measure by the maltster—which eventuate in the production of a pleasant-flavoured, friable, sweetish substance—malt, from the stubborn and almost tasteless barley: the transformation, in fact, of a substance which yields very little matter soluble in water into one which is capable of giving—when properly infused in the mash-tun—a richly saccharine liquid or 'wort.' Science and its handmaid, chemistry,

tell the maltster the times of duration for the various processes and the temperatures at which they should be conducted, and many other important matters. The microscope, which has been employed to demonstrate the minute anatomy of the barleycorn, is capable of showing the maltster various structural changes in the corn corresponding to its gradual conversion into malt. Associated also with the science of malting are bacteriological considerations relative to the purity of the air of the neighbourhood in which the maltings are situated, and the presence of floating dust. It is very difficult, in fact almost impossible, to avoid mould and other deteriorating influences when the air of the maltings is laden with dust from internal or external sources.

The maltster having exercised his skill, the brewer's part in the proceedings comes under consideration. Malt is not an article which can be made of practically invariable composition; it needs must vary according to the kind of barley used, the time of year it is malted, the period of storage of the malt, &c., and unless the brewer—which is seldom the case—is able to draw his supply of malt from a very large uniform stock, he finds himself dealing with a material that deviates not a little from a normal behaviour in the brewery: for instance, the fermentations may become irregular, that is, they may take a longer or shorter time than usual, and the resulting beer may taste either too sweet and full, or be, on the other hand, thin and poor. Again, the quantity of yeast that is yielded by a wort entering on the latter stages of fermentation may be scanty in quantity, and weak when used to set and ferment a subsequent brewing. Scientific knowledge has been applied to meet such cases as, for instance, by the careful analysis of malt samples prior to the use of the malt in the brewery. The information thus obtained enables the brewer to vary the conditions of the process so as to obviate the possible defects alluded to. Next to a close knowledge of the nature and character of the malt, and a feeling of assurance that it is fitted to produce sound and well-tasting beers in conjunction with a suitable water and a well-considered process, the brewer's chief anxiety should be to keep the 'plant' scrupulously clean; for if there be neglect in this direction the yeast soon suffers, and instead of the microscope showing the healthy living cells of normal yeast (Saccharomyces Cerevisiae), it discloses other organisms, such as microbes or bacteria, and unless this state of things can be arrested whilst still incipient, the beer brewed under these circumstances will develop unsoundness. So important also is

the matter of cleanliness of surroundings that in the building of new breweries a site is preferably chosen in the open country away from the aërial contamination of large towns. Enough has perhaps been said to indicate that a well-equipped chemical and biological laboratory is almost a necessity in the modern brewery, and it may be said here that the investigations conducted in it do not stop at the beer as it is filled into the sale casks, for, by the use of suitable apparatus, it is possible to submit small specimens of the brewings to artificial conditions which will give one a fair idea of what their behaviour in the cask will be.

[Since the above was penned the episode of the occurrence of arsenic in certain beers has brought the question of the scientific control of the brewing process very strongly to the fore. It has demonstrated the fact that scientific supervision is largely exercised, that it requires to be more closely exercised; that reasonable supervision will not secure immunity from that which is accidental in its nature; that brewers cannot be too careful in the choice of the materials they employ. The arsenic trouble has established in addition the fact that minute quantities of a poisonous substance can come in from hitherto unsuspected sources; and, last but not least, it has demonstrated that where malt and hops are the chief ingredients in a beer, and where all possible care has been exercised in the selection of these orthodox ingredients, the public has had little or no cause for apprehension as regards the purity of the beverage.]

Malting.

Malt is such an indispensable adjunct of the processes of brewing and distilling, that it is hardly possible to obtain a clear view of the scientific principles which govern them without devoting some attention to the produce of the maltster's skill and science.

Malting consists of an artificially controlled germination of grain, which in the case of the brewer is barley, but may be rye, oats, &c., in the case of the distiller.

The following are the salient features of the malting process:—

(*a*) Screening away from the barley, by a properly contrived machine, light corns, seeds, stones, dust, and other extraneous matters.

(*b*) The screened barley passes to a vessel or receiver, termed the

cistern, in which the vital processes are set in action by steeping in water for a period varying from the fairly usual one of forty-eight hours to as much as seventy-two hours. The barley is finally drained from the excess of water.

(*c*) The steeped grain is thrown out of the cistern on to the malting floors and gathered into a levelled heap called the couch; here it remains some two days, and germination commences and is visible on the second day.

(*d*) Successive turnings and thinnings out of the 'pieces' on the floors take place on the third and fourth days as growth becomes more and more vigorous and the temperature tends to rise; a notable increase in the length of the rootlets is visible, and traceable lengthening of the plumule or acrospire at the back of the corn.

(*e*) Fifth and sixth days after removal from steep: some slackening of growth from loss of moisture ensues; the 'pieces' are generally sprinkled with water on these days, which first retards growth a little and then accelerates it. Seventh and eighth days: further growth of the 'pieces,' loss of moisture, but great activity in growth.

(*f*) Ninth and tenth days: withering to make the green malt ready for going on the kiln—a period during which a further percentage of water is lost, and the malted grain becomes perceptibly drier to the hand, whilst the rootlets perceptibly wither and shrivel.

(*g*) Gathering the green malt on to the kiln, called 'loading,' and drying it off at the required temperature, called drying and curing. This operation generally takes four days, but is often less on improved modern kilns.

(*h*) Garnering for subsequent use, i. e. storage in garners, or in bulk on upper floors of maltings in spaces designed for the purpose.

Most cereals can be malted, but barley in its general attributes is the grain best suited for the purpose. Barleys which are destined for the brewer's use as malt should be of good quality, raised from good seed grown on favourable soils under climatic conditions which, unfortunately, are not always realized in this country. The reaped barley should have been well 'sweated' in stack, and then so carefully threshed and dressed that it is reasonably free from half-corns, foreign seeds, light grain, stones, dirt, &c., and with the husk neither split nor

torn at the ends of the corn[1]. So great is the variety of kinds and qualities of barley, English and foreign, now offered for malting purposes, that it is almost easier to say what a barley should not be than to try to describe the appearance of good samples. The following points may, however, be noted:—The barley should look bright and clean and well wrinkled, for the latter appearance is a useful indication of good harvesting and storage. The skin should not be very thick nor the grain high coloured, for although barleys of this description may make very fair malt for mild ales, a delicate, pale, thin-skinned barley is desirable for pale ale malt. If the barley carries too much moisture it is usually evidenced by a peculiar clinging feeling when a handful is grasped and the fingers slowly opened; when really damp, a mouldy smell manifests itself in a greater or less degree. Such barleys, if not very far gone, can be brought into a satisfactory state by drying them in bulk on the malt-kiln at a temperature of 120° F.; this removes the excess of moisture and brings the latter to a normal figure, say about 12 per cent. Moreover the kiln-drying has the effect of greatly improving the capacity of the barley for germination; it is advisable, however, to keep the barley for a week or ten days to cool off before steeping it.

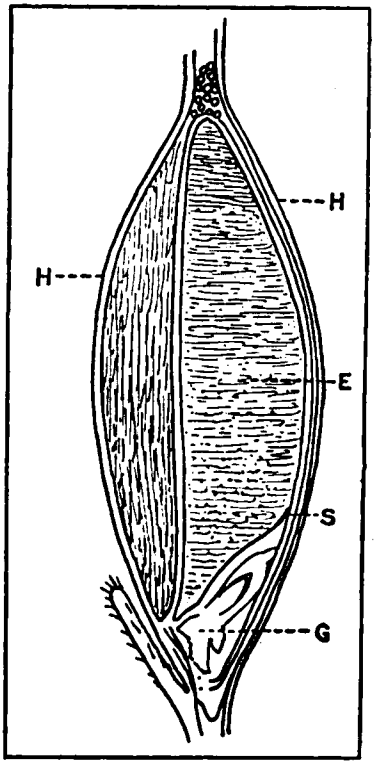


FIG. 11.—Longitudinal section of the Barleycorn through the furrow.

Without attempting to enter into the minute anatomy of the barleycorn we may, for our purposes, regard it as consisting of

[1] Samples of barley otherwise of good quality are often greatly reduced in value by want of care in the adjustment or proper repair of the working parts of the threshing-machine. The defects produced in the barley injuriously affect the malt made from it.

three chief parts—the germ G, the endosperm E, and the husk H. See Fig. 11.

The germ portion is the seat of vitality and contains the rudiments of the plumule or acrospire and the radicles or rootlets. This portion of the corn consists mainly of nitrogenous substances. We may regard the endosperm, or starchy portion of the corn, as the store-house from which the germ draws its supply of nourishment as growth proceeds. The germ is definitely divided from the endosperm by a plate or shield of columnar structure, known as the scutellum (Fig. 11, S), and the germinal portion of the corn may be dissected away from the endosperm and yet retain its vitality. We need not give the husk any special attention, though structurally it is very interesting. It consists mainly of cellulose.

The endosperm contains starch granules of two different sizes, also other carbohydrates and nitrogenous substances, the whole interspersed with mineral matter, mainly phosphates. The various matters of the endosperm are somewhat divided up by compartments, consisting probably of the less resistant form of cellulose known as hemi-cellulose. Where the husk folds into the corn at the furrow there is a channel (see transverse section of the corn, Fig. 12) running nearly the whole length of the corn, which doubtless very much aids the transfusion of liquids from the germ to the endosperm and vice versa during the germination. Very little of the germ or of the endosperm of barley is soluble in cold water; what there is consists of soluble albumen, sugars, phosphates, &c. This brings us directly to the process of steeping, which we may now enlarge upon somewhat.

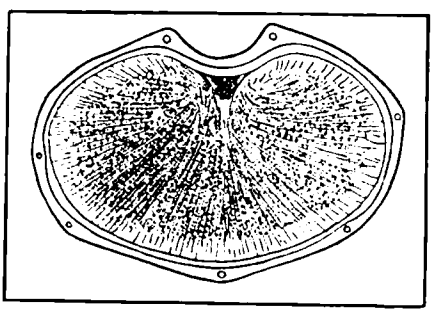


FIG. 12.—Transverse section of the Barley-corn.

The screened barley is run into a receiving vessel, with drainage channel and plates, known as the cistern, and cool and reasonably pure water is run on till the barley is completely immersed. The water is drained away at the end of about twenty-four hours, the wet barley is washed by running some water through, and a second 'tide'

of steep water is run in, in which the barley is left for another period of twenty-four hours. Most barleys are sufficiently steeped in forty-eight hours, but very hard barleys may require as much as seventy-two hours. The barley absorbs nearly 50 per cent. of its weight of water during the steeping, and swells from 16–18 per cent. of its original volume, at the close of the steeping the last water is run off and the wet barley is drained. Some light corns and floating extraneous matters are skimmed off the steep from time to time.

There is a general consensus of opinion that a moderately hard water is preferable to a soft one for steeping purposes, since very soft waters are liable to extract too much matter from the barley, and a uniform temperature, though hard to secure, is also a very desirable thing. About 55° F. is a very good temperature to work to.

Steep water extracts on the average about 1·5 per cent. of the weight of the barley, the substances dissolved out being both organic and inorganic, e.g. carbohydrates (sugars, &c.), colouring matter from the husk, soluble albumen and other nitrogenous compounds, and mineral matters, especially potassium phosphate. The nature of these substances causes steep water to be a highly putrescible liquid, especially the water of the first tide, which naturally carries much more matter in solution than the second water. The rapidity of decomposition is greatly enhanced by the fact that the steep water is loaded with bacteria, mould-spores, &c., derived from the exterior of the barley, and it is of no little importance that these organisms should be removed to the extent they are, as the danger of mould—the maltster's chief foe—on the growing pieces is greatly lessened thereby.

The barley, being now washed fairly clean and carrying enough water for effective germination, is thrown out of the cistern on to the malting floors, being gathered into a levelled heap, about 14 inches deep, called the 'couch.' The temperature rises a little, and in this somewhat limited contact with air the germinal parts of the corn begin to develop, till it comes into a state of visible growth called 'chitting,' the rudimentary rootlets being perceptible, whilst the plumule or acrospire begins its gradual progress up the back of the corn between the husk and the starchy portion of the grain (Fig. 13). This marks the first stage of growth, extending over two days, at the end of which time the original 'couch' has been 'broken' and thinned out by shovelling, and becomes a 'piece' or 'floor.' The temperature of the couch should not have exceeded 65° F. nor fallen below 50° F.

The growth proceeds rapidly in the pieces and they require rather frequent turning—at least twice a day—to ensure a sufficient supply of air for the respiration of the growing corn, and such uniformity as

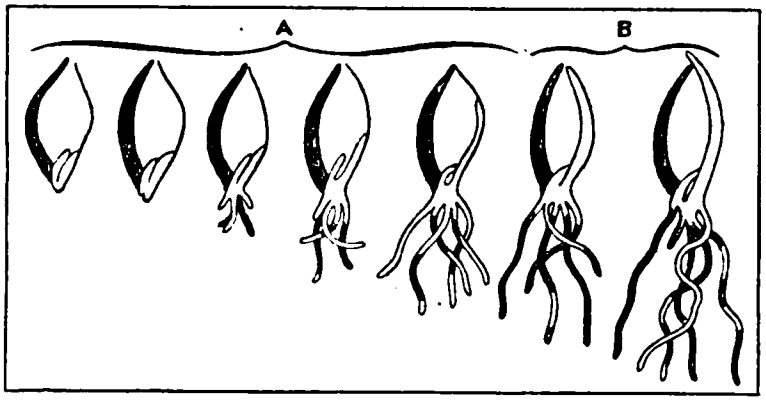


FIG. 13.—Progressive growth of the Acrospire and Rootlets. A. 1st to 9th or 10th day on the 'floors.' B. Overgrown Corns.

regards aeration and temperature, as will result in the corns developing rootlets and acrospire in a similar way, for regularity in growth is one of the chief essentials in good malting. We are at a point now when some five days have elapsed since the barley came out of steep; the growth has become very active, but is tending to flag on account of the loss of water, caused partly by evaporation and partly by the chemical and physiological changes going on in the germinating grain. We may pause to inquire what these changes mainly are. They may be grouped as follows:—

1. The production of enzymes (in addition to the small quantity of saccharifying diastase originally present in the barley).
   - (*a*) Diastase which can liquefy gelatinized starch and can even act on granular starch under certain conditions.
   - (*b*) Cytase, capable of dissolving hemi-cellulose—the compartmental matter of the endosperm.
   - (*c*) An enzyme of the nature of peptase which can dissolve albumen (proteolytic action) and give rise to diffusible nitrogenous substances allied to the peptones.

2. The production of sugars, or, more accurately, a large increase in the quantity of sugars, formed mainly at the expense of the starch and

its congeners. This modification and alteration of the starch takes place especially in the vicinity of the acrospire, portions of the granules being eroded away: it is not improbable that cytase plays some part in this action as well as diastase. The destiny of the sugars so produced is, doubtless, to nourish the germ, to which they are conveyed by the natural circulation of the liquids in the barley-corn; only under the conditions of malting the sugars are produced much more rapidly than the germ is able to assimilate them, and produce a corresponding amount of fresh tissue—acrospire and rootlets.

Kjchldahl gives the following figures as representing the increase of diastatic (starch-converting) power during the progress of the germination:—

1st day	70	5th day . . .	150
2nd „	73	6th „ . . .	190
3rd „	80	7th „ . . .	220
4th „	105	8th „ . . .	226

[These numbers must be taken as relative, for they do not appear to correspond with Lintner's scale and mode of expression.]

As regards the formation of sugars—we may take it that barley on the average contains a little over 2 per cent. of sugar (perhaps nearer 3 per cent. on the dry substance, for barley normally contains some 12–13 per cent. of water); the sugars are cane sugar, fructose, and glucose, and sometimes raffinose. The finished malt seldom contains less than 8–9 per cent. of already formed sugars, and generally contains nearer 12 per cent. The following figures, which are the means of results obtained by Cornelius O'Sullivan, will illustrate the matter very well:—

Sugars in Malt.

	Per cent.	The same calculated on a normal content of sugar of 12·9 per cent.	
Cane sugar	4·40	5·20	The cane sugar is some 2 per cent. higher than is generally found in the wort after mashing the malt. It becomes inverted to the extent of about 2 per cent.
Maltose	3·15	3·72	
Dextrose	2·25	2·67	
Levulose	1·10	1·31	
	10·90	12·90	

We will now return to the malting process proper, having left it at the sprinkling stage, the fifth and sixth days. The growth on the floors is allowed to proceed till the ninth or tenth day, dating from the barley coming out of steep. By thinning out, thickening up, turning, &c., the growth of the acrospire and radicles is made as even as possible, and the temperature of the pieces kept down or brought up as is rendered necessary by changes in the weather, direction and force of the wind, &c. The physiological and chemical changes still go on actively. The respiration continues, carbonic anhydride being evolved in considerable quantity. A not inappreciable amount of starch—from 4-6 per cent.—is wholly lost during the germination, the end products being most probably water and carbonic anhydride. Some of the oxygen absorbed seems to become fixed, since Cuthbert Day found in his experiments that more oxygen was absorbed during germination than was accounted for by the oxidation of the carbon to carbonic anhydride.

The germination of the grain from the earliest stages—say the second day—is attended by the development of odours of a more or less ethereal kind: if the growth is progressing favourably, the odours are rather pleasant than otherwise; any distinct unpleasantness of smell is a matter to be taken note of, for it may mark overheating, incipient mould, arrested development, and so forth.

During the period of energetic growth, it is well if the thermometers —one of which should be on each floor at a height which suffices to give the air temperature accurately—do not indicate a temperature exceeding 55° F., certainly not exceeding 60° F., and the pieces should be at a temperature only a few degrees above the air temperature. This is to secure an equable growth at a moderate temperature, for rapid growth at elevated temperatures (technically termed 'forcing'), although it may conveniently shorten the period necessary for converting barley into malt, is very much to be deprecated, if not condemned, for forced malts do not give satisfactory results when brewed. This is due most probably to the fact that 'forced' malts carry a high percentage of soluble phosphates and less diffusible nitrogenous substances available as yeast food. A high percentage of sugars is known to obtain in such malts, but there is no particular reason why this in itself should be harmful. Morris and Moritz in their well-known Textbook on Brewing give the following figures, which will be useful in this connexion:—

	A. Malt from barley rapidly germinated.	B. Malt from barley grown slowly.
Respired	8·36	6·38
Malt (exclusive of rootlets and acrospire)	83·09	85·88
Acrospire	3·56	3·09
Rootlets	4·99	4·65
	100·00	100·00

The ready-formed sugars in A were very much higher than in B.

The next stage in the malting process is that in which the reduction in the percentage of water carried by the germinating grain begins to make itself visible in a drying up of the rootlets and a perceptibly drier state of the whole 'piece.' This is the beginning of the withering stage, and is associated with the eighth or ninth day. The physiological activity in the corn is still very considerable, but its manifestation is more especially confined to the acrospire, which may grow very fast, and if the piece be carelessly handled may even grow out beyond the corn. In any case the production of sugars is rapid at this stage, and if the acrospire grows up too far, a bitter taste may become apparent in the malt. A growth of acrospire of three-fifths to three-fourths the length of the corn is a desirable thing to aim at in the finished malt. The object of the maltster is to facilitate the withering, and the further loss of moisture which accompanies it, by thickening up the pieces and letting the temperature rise somewhat, so that the malt shall not go to the kiln wet, but in a state in which the grain when smashed has a kind of damp chalkiness. This state is consistent with a content of water of about 30 per cent. The temperature of the withering pieces should not exceed 80° F., and it is better if they can be worked below this temperature. This stage of the process extends over one or two days as occasion requires, and the total time on the floors is nine or ten days from throwing out of steep. The malt is now ready to be loaded on to the kiln, and to be dried and 'cured.' It is desirable that the load on the kiln floor should not exceed 8 or 10 inches, otherwise it is difficult to get the moisture away, as it tends to condense in the upper part of the layer. The chief objects in drying the malt on the kiln are the following :—

(*a*) To remove the large excess of moisture, and bring it down to 3 per cent. or under.

(*b*) To absolutely stop the germination.

(*c*) To destroy a considerable amount of the diastase, the diastatic power being too high for brewers' purposes. To somewhat modify the action of the remaining diastase.

(*d*) To produce the so-called 'caramelization products,' which impart the nice biscuit flavour of well-cured malt. (These are probably derived from the carbohydrates and the soluble albuminoids.)

(*e*) To fix the composition of the malt, and thereby bring it into a state in which it can be stored without fear of rapid deterioration.

The green malt being on the kiln, a gentle, well-regulated heat is applied for the first twenty-four hours, the temperature being allowed to rise gradually to 120° F. As good a draught as possible is secured, so that the moisture arising as vapour may be carried away. During the next period of twenty-four hours the temperature rises gradually to about 140° F.—still with a full air current. In the next twenty-four hours the temperature is allowed to rise to about 170° F. for mild malt and 160° F. for pale malt, the amount of air admitted to the fire space, and so to the floor, being much less. The kiln, which has been turned at the end of the first twelve hours, and at shorter intervals afterwards, is also trampled to break away the 'culms,' as the dried rootlets are called. During the first part of the last twenty-four hours (seventy-two hours having already elapsed) the heat rises to what is called the curing temperature, which is about 180° F. for pale malt, and some 10° higher for mild malt. Although the whole of these temperatures may be regarded as usual and average ones, they are subject to very considerable variations according to the structure of the kiln. On some modern kilns it is possible to dry off malt quite 20° F. higher than on old-fashioned kilns, and get a malt of the same character. Again, the draught and ventilation have been improved so much that 25–30 per cent. of the time formerly required will suffice to dry and cure the malt. The temperature is maintained for not less than six to eight hours at the maximum; it is then 'rooked' into a heap in the middle of the kiln, and left at a falling temperature till the expiration of the fourth day (ninety-six hours), at which time its temperature would be about 120° F. The proper adjustment of the temperature and draught on a kiln is of the very first importance, and reliable thermometers are indispensable. Where the through draught on a kiln is strong and equal, it is possible not only to dry off malt more quickly, but to 'cure' it at a higher temperature than where

the draught is bad, without gaining an undue amount of colour. The aim of the maltster should be not only to produce an evenly grown malt, but an evenly cured one as well.

Above all things malt should be tender, i. e. easily crushed and completely friable. The disappearance of the cellulose compartments during germination is the direct cause of this state, subject to careful removal of the moisture. The early stages of the kilning process require much care, for if the moisture be not driven off expeditiously, changes go on in the green malt which lead directly to the great fault of 'steeliness.' It is necessary that the excess of moisture be removed whilst the temperature is still comparatively low, and only careful firing and a good draught will ensure this. Grüsz has latterly paid much attention to the subject of steely malt, and we may give his results as follows:—Steeliness consists in the starch granules in the endosperm becoming embedded in a mass of gummy matter containing a large proportion of reducing sugars; this state of things is most probably due to the action of some enzyme of the nature of cytase, the action of which is permitted by the moist state of the corn and greatly fostered by the elevated temperature which it has reached on the kiln. Grüsz's analytical results are very instructive, viz.:

	Slow drying of malt in—		Quick drying of malt in—	
	Moist air.	Dry air.	Moist air.	Dry air.
Invert sugar . . .	12·5	7·37	6·6	6·5
Cane sugar . . .	11·5	6·37	9·0	6·3
Total ready-formed sugar	24·0	13·74	15·6	12·8

Grüsz assigns the following changes as marking different periods of kilning:—

(*a*) Continuation of the growth of the germinal parts (the embryo), removal of carbohydrates, translocation of sugar from the embryo to the endosperm, and continued respiration.

(*b*) A continuance of enzymic action (diastase, cytase, &c.).

(*c*) Continuance of enzymic action, coagulation of albuminoids, with change of the hemi-cellulose [it is here that the gummy matter is most probably produced; restriction of diastatic power takes place].

(*d*) Roasting is completed.

The temperature at which the malt is finished is a matter which affects in a most important way the composition of the wort resulting

from it. We will reserve this, however, as regards detail, until we come to the mashing operation in Chapter X. It will suffice for our present purposes to say that the higher the drying temperature the more colour the malt will carry, and the higher coloured and the less fermentable will be the resulting wort; whilst the flavour of the caramelization products will be the more perceptible in the malt wort. It will be gathered from this that the malt is dried at a temperature corresponding with the kind of beer it is intended to produce. The following maximum drying heats may be taken as approximating to what is required for the class of malt indicated :—

Pale ale malt . .	180° F.
Mild „ . . .	190° F.
Amber malt . . .	250° F. (for strong ales and porters)
Brown „ . . .	300° F. (for stouts and porters)
Black or patent malt .	700° F. („ „)

The following figures given by E. Prior show remarkably clearly the effect of temperature on diastatic power; the figures are doubtless on Lintner's scale :—

Temperature F.	Diastatic power.	Temperature F.	Diastatic power.
101° . .	134	178° . .	66·5
123·5° . .	85·2	190° . .	47·2
144·5° . .	85·0	201° . .	26·4
156° . .	72·6	212° . .	15·1
167° . .	72·6		

The following is an exposition of the relationship of the chemical composition of barley to that of malt :—

	Barley.		Malt.	
	Normal.	Water-free.	Normal.	Water-free.
Starch and other carbohydrates, mainly insoluble in water	56·0	63·6	50·8	52·3
Sugars	2·3	2·6	12·9	13·3
Products of the caramelization by kiln heat	. . . .	. . . .	2·5	2·6
Cellular matter . . .	12·5	14·3	13·5	13·9
Fat	2·4	2·7	2·0	2·1
Nitrogenous substances, albuminoids, and amides	12·0	13·6	13·0	13·4
Ash	2·8	3·2	2·3	2·4
Water	12·0	—	3·0	—
	100·0	100·0	100·0	100·0

Most of these components have been dealt with in some detail. A few words may be added about the albuminoids. In barley, barely one-fourth of the nitrogenous substances are soluble in water, cold or hot. In the case of malt about one-half of the nitrogenous substances are soluble in water, taking temperatures throughout a range of 15·5° C. (60° F.) to 68° C. (154·5° F.). Both barley and malt contain albumen soluble in cold water, but coagulable at 70° C. (158° F.), and in each case, at least a portion of this is albuminoid matter of the nature of diastase. The malting process no doubt results in the malt containing a portion of its soluble nitrogenous matter as substances of the nature of peptone.

COMPONENTS OF THE ASH OF BARLEY AND MALT.

	Barley (3·2 per cent. on dry barley)	Malt (2·4 per cent. on dry malt)
Potash (K_2O)	21·6	16·1
Soda (Na_2O)	2·1	2·9
Magnesia (MgO)	8·2	7·5
Lime (CaO)	2·6	4·4
Oxide of iron (Fe_2O_3) / Alumina (Al_2O_3)	1·1	1·6
Phosphoric anhyd. (P_2O_5)	34·8	33·2
Sulphuric anhyd. (SO_3)	1·7	0·7
Silica (SiO_2)	27·0	33·1
Chlorine (Cl)	0·9	0·5
	100·0	100·0

For purposes of comparison with the above, and as giving some further indication of the inorganic substances removed by steeping, we append an analysis of the ash of steep-water. About 10 per cent. of the original inorganic matter is removed by steeping.

Potash (K_2O)	54·80
Soda (Na_2O)	2·60
Magnesia (MgO)	4·20
Lime (CaO)	2·10
Iron oxide (Fe_2O_3) / Alumina (Al_2O_3)	0·06
Phosphoric anhyd. (P_2O_5)	11·70
Sulphuric anhyd. (SO_3)	4·30
Silica (SiO_2)	2·80
Sodium chloride (NaCl)	12·70
Carbonic anhydride	4·74
	100·00

It will be seen at once how important a part potassium phosphate constitutes of the matters withdrawn. The analysis indicates that some of the potassium is in the state of carbonate, having been formed by the interaction of lime salts of the water (carbonates, sulphates, &c.) with potassium phosphate in the barley. Lime has replaced the potassium, and other salts have entered in from the steep-water—chlorides, &c.

It has been said earlier in this chapter that one of the chief enemies of the maltster is mould. Two or three kinds of mould are known to occur on the growing barley, but by far the commonest is the ordinary blue-green mould, Penicillium Glaucum. If this mould gets a fair hold upon a growing piece, and the temperature be elevated or the weather close and humid, it may spread with wonderful rapidity; it is usually apparent first on half-corns, corns with split husk, and on those trodden and smashed during the operations of the working maltster. F. E. Lott's conclusions as to the effect of mould on malt[1], based on a series of experiments, are the following :—

1. The peculiar flavour so strongly apparent on biting and chewing malt containing mouldy corns is not conveyed to the wort obtained on mashing the malt, nor to the beer obtained by fermenting the wort.
2. The colour of the wort is increased by the mould.
3. The total extractible matter obtained by mashing or infusing the malt is lowered.
4. The ratio of sugars to non-sugar is lowered, that is to say, there is less fermentable matter in the wort, and consequently less alcohol is formed.
5. The amount of inactive matter, or non-ascertainable carbohydrates is not increased, but there is a larger proportion of nitrogenous substances.
6. The acidity of the extract from mouldy corns is markedly higher [this may be due partly to organic acids, partly to acid phosphates].
7. The diastatic activity estimated by Lintner's method is higher in the mouldy than in the non-mouldy corns.

It may be added that although the wort from mouldy malt-corns does not taste mouldy, there is a definite want of flavour, caused doubtless by destruction or modification of certain substances which

[1] *Trans. Fed. Inst. Brewing*, 1899, vol. v, p. 2.

give the normal agreeable flavour to malt and malt extract. It behoves the maltster to use every effort to prevent the growth of mould on the floors, and the following points are some of the more important ones for the maltster to take note of, if he wishes to produce a sound and good malt:—

Careful brushing and screening of all barleys.

Regular temperature of the steep-water, summer and winter (that is, if malting be carried on at all in the early summer months).

Liberal supply of water in steeping and for washing through.

Temperature of the air over the 'pieces' as uniform as ventilation will permit.

Temperature of the 'pieces' low and uniform.

Scrupulous cleanliness on the floors, and care exercised not to smash corns; also not to create dust in the neighbourhood of the malting floors.

Position of the maltings, if possible, in a pure, free air, and away from road dust.

Whole of the germinating process short of the withering, slow and cool, and the withering not unduly hurried.

Great care in the early stages of kilning to get the moisture away before the temperature runs up.

The malt warm from the kiln is usually screened from the culms by wire screens against which it is thrown (sometimes it is stored with the culms), and then it is garnered in bulk in bins, which are usually spaces partitioned and boarded in wood, closed in at the top, and provided with sliding doors for the withdrawal of the malt as required. If the malt is to be stored for a period of many months it is advisable that the bins are lined with thin sheet metal—zinc, copper, or tin-plate.

It is most desirable during storage to protect the malt from air currents, as it very readily absorbs moisture and becomes 'slack.' Coming from the kiln it should not carry more than 3 per cent. of moisture, and 2·5 per cent. is even better; when the moisture exceeds 5 per cent. the slackness is apparent, the malt being dull when bitten instead of being crisp and tender. Slackness soon affects the flavour, the nicer qualities disappearing as the percentage of moisture increases. The malt should be stored or garnered at least one month, and better five or six weeks, before it is used for brewing. When it is required in the brewery it is bushelled up into sacks and sent to the malt room of the brewhouse.

The following figures show the relationship of the original barley

to the finished malt from what may be termed the practical maltster's point of view:—

100 quarters of barley (4 bushels per quarter) yield:—
Screenings or light barley, 3·75 quarters (100 − 3·75 = 96·25).
Barley malted, 96·25 qrs. yields 101·5 qrs. of malt and 41 lb. to the bushel
or 99·0 „ „ „ 42 „ „ „
and 3·5 per cent. of culms.

and from above—

100 lb. of barley = 77·0 lb. of malt
loss = 23 per cent. made up as follows:—

Moisture . . .	10·5	difference between barley 13 and malt 2·5
Loss by steeping . .	1·5	
Loss by respiration .	6·5	
Rootlets or culms .	3·5	
Floating corns skimmed off steep . . .	1·0	
	23·0	

We will conclude the chapter by giving a very systematic and useful method schemed out by F. E. Lott for making a hand examination of malt, and so obtaining a considerable amount of information which may be supplemented if desired by chemical analysis.

1. Appearance of the corns looked at *en masse*, say one or two thousand corns spread evenly on a table in a good diffused light, but not in strong sunlight. Note the following points:—
   (*a*) Size. Are the majority of the corns large or small, thin or plump, thin or thick skinned?
   (*b*) Grading. Are they evenly graded or badly graded? Are there many foreign substances, such as seeds, peas, wheat corns, oats, stones, &c.?
   (*c*) Colour. Is the malt dull or bright, pale, medium, or dark?
2. Appearance of the corns examined in detail.
   (*a*) Faulty corns. Pick out and note the number, whether many or few, discoloured, broken, half-corns, &c.
   (*b*) Mould. Whether present or not, especially if only on broken corns or not; if mainly blue mould, or if red or black mould on any corns.
   (*c*) Growth of plumule. Regular or irregular, examine carefully about fifty corns and classify.

(i) Undergrown with acrospire up to half-way.
(ii) Well grown with acrospire up to three-fourths.
(iii) Overgrown with acrospire up or out.

3. Taste, &c. Thoroughly masticate about fifty corns. It is well to avoid including any mouldy corns in this test, as they usually give such a pronounced flavour that they obscure the general flavour of the sound corns forming the bulk of the malt.

(*a*) Condition; i.e. if slack or not: or if some few odd corns are soft and the remainder are in good condition. Experience will enable one to estimate the moisture to within 1 or 2 per cent.

(*b*) Steely or hard-ended corns.

(*c*) Flavour. If markedly sweet the percentage of ready-formed sugars is probably high, and the malt probably 'forced'; if raw or like barley, under-curing is probable; if with an after bitter taste the malt has been probably overgrown (compare 2. (*c*) iii); if markedly flavourless the barley has most probably been not properly matured before malting, and the malting has been inefficient.

(*d*) Curing. This may be judged fairly accurately by the taste.

(*e*) Firing or kilning. The amount of fire-heat to which the malt has been subjected may be approximately estimated by the flavour, and this is not necessarily a proof of curing, as a high-dried malt may occasionally be an under-cured one, although this is unusual.

A nice biscuity flavour shows proper firing even for pale malts, and high-dried malts taste even more fully of the caramelized bodies they contain.

4. Sinkers test. 100, or better 200, corns are counted out and placed in the bottom of a tumbler, this is then filled three parts full with clean water, stirred up at once, and the floating corns removed. The total number of sinkers is then noted, and they are taken out and individually tested by biting. They may be classified as follows:—

(*a*) Unmalted barley corns. These are quite hard and difficult to bite.

(*b*) Partially malted corns. These break, but have more or less hard ends; they may be subdivided into half-malted and three parts malted.

(*c*) Soft corns. These are malted corns with an exceptionally high percentage of moisture.

If the sample of malt is slack there may be a considerable number of corns that will not float which do not come under any of these three headings, whereas in a well-cured malt in good condition any sinkers will come in divisions (*a*) or (*b*).

5. Smell. Any mustiness or unpleasant flavour, due to faulty storage in sacks or damp places, may easily be detected by carefully smelling a handful of the malt.

Pneumatic Malting.

For quite twenty years past attention has been directed both in this kingdom and on the Continent to the possibility of improving the malting process by so modifying the methods of working as to make more economical use of the space in maltings, to reduce the labour necessary, and also to economize in the fuel used for drying and curing the malt. System has succeeded system, and improvement has been grafted on to improvement, until there are, at the present time, certain well-defined methods of procedure which seem to have some element of permanency in them. Pneumatic malting in the modern acceptation of the term denotes that the steeping, the germination, and the withering of the barley are carried on in one and the same vessel, usually a cylinder capable of being rotated on an axis at a controllable speed, and supplied with air which can be rendered moist or dry as required. Arrangements are also made by which the temperature of the air supply can be regulated, and in some cases the cylinder is caused to rotate in a trough containing water which can be kept at a desired temperature so as to govern the temperature of the contents of the cylinder.

Inasmuch as daylight is not necessary for the germination of the barleycorn it is plain that this operation may be effectively carried on in a closed vessel if only the supply of air, the withdrawal of carbonic anhydride, the addition or subtraction of moisture, and the turning of the growing barley be properly adjusted and carried out in a manner parallel to that which would take place in a malting conducted on the ordinary plan. In a very large measure all these things are possible, but it requires a more highly skilled class of labour than can be expected from the ordinary working maltster, and perhaps this is the chief reason why the success of pneumatic malting is not

so fully assured as theoretically might have been expected. Another outcome of economical motive is the so-called double kiln, which, as the name implies, is an arrangement of one kiln-floor immediately above another, the idea being that the surplus heat which ordinarily escapes from the kiln is utilized to dry a green piece perhaps two days younger, which is loaded on to the upper kiln and remains there two days till the lower kiln is unloaded, when it takes the place of the malt withdrawn, another green piece going on the upper kiln. This method unquestionably effects a great saving of fuel, but the weak point is that any variation in the temperature of the lower kiln is bound to have some corresponding effect upon the upper kiln, consequently the production of tender, well-cured malt is rendered more difficult. On the Continent the method of direct heating of the kiln by combustion of coke and anthracite[1] seems to be giving place to the use of flues or hot-air pipes in contact with or proximity to the germinated grain which is to be dried. Such a method should permit of much better control of heats, and economy in the use of fuel. There is much to be said in favour of the usual method of drying over a clean fuel such as anthracite, but if—as appears probable—equally good results are obtainable by flues or pipes, it can hardly be doubted that the newer method will eventually supersede the older one.

In connexion with more modern methods of malting, mechanical turning of the malt on the kilns is worthy of mention, not only from the economy resulting, but especially from a humanitarian point of view, work on the kilns at the higher temperatures being, on account of the dust, the heat, the sulphurous acid, &c., a trying and unhealthy part of an occupation which is in most other respects healthy, interesting, and by no means devoid of scope for skill and intelligence on the part of the working maltster.

[1] At the present time coke has fallen into disfavour as a fuel for malting purposes, as it has been found to give malts containing traces of arsenic, a fault which anthracite—speaking generally—is not guilty of.

CHAPTER X.

BREWING.

WE will commence—as in the case of the malting process—by taking a brief survey of the chief operations of the brewing process. They run as follows:—

1. Crushing or grinding the malt as it comes from the maltings, by means of a malt mill.

2. Passage of the ground malt (grist) to a receptacle called a grist-case or grist-hopper situated over the mash-tun. The ground malt is stored in this receiver for a few hours under conditions which prevent any free exposure to air.

3. Mixing or 'mashing' the grist with hot water into the mash-tun by means of suitable mechanical aids, which ensure thorough mixing of the grist and hot water (liquor). The mixture thus made is termed the mash.

4. The withdrawal, after an interval of time, of a large portion of the resulting sweet 'wort' by taps connected with orifices in the bottom of the mash-tun: these orifices are under a perforated strainer called the 'false bottom': on this false bottom the 'goods' rest.

5. Sprinkling the partially exhausted 'goods' with hot water (liquor) by means of a rotating sprinkler called a 'sparger': the operation is termed 'sparging,' and the object is to extract and wash the soluble matters from the 'goods,' which at the close of the operation are called 'grains.' The wort thus extracted may pass to a vessel underneath the mash-tun call the underback.

6. The sweet wort is sent from the underback, or passes direct from the running-off taps to the wort copper, where it is boiled by fire or steam heat in contact with the hops, which are emptied from the sacks in which they have been weighed up for the brew into the sweet wort in the copper.

7. After a thorough boiling the contents of the wort copper are discharged into a vessel provided with a strainer, called the hop-back: here the 'bitter wort' is strained away from the hops, and is conveyed

—usually by pumping—to the wort receiver, or cooler as it is more generally termed. The spent hops in the hop-back are either returned to a weaker wort in the wort copper to be reboiled, or they may have hot liquor sparged over them to wash out adherent extract, or they may be pressed. The 'spargings' or pressings may go to the cooler or into a following copperful of wort.

8. Partial cooling of the scalding-hot wort takes place on the cooler, and most of the suspended matters, not filtered off by the hops in the hop-back, are deposited as a buff-coloured, somewhat flocculent deposit called 'cooler grounds.'

9. Complete cooling to the temperature at which it is intended to collect the wort in the fermenting vessel, is secured by running the wort over a refrigerator, a powerful cooling apparatus constructed so that the wort travels over a system of pipes or compartments through which cold water circulates. It is so made that the coldest water meets the coldest wort, and the cooling water passes away from the refrigerator at a temperature nearly equivalent to that at which the wort is entering.

10. Collection of the bitter wort in the fermenting vessel, and addition, at an early stage of the collecting of the wort, of the yeast used to excite fermentation: the latter operation is termed 'pitching.'

11. Commencement, development, and progress of fermentation to a point at which the yeast which tends to come to the surface[1] is either removed by skimming it off at intervals, or caused to separate by dividing the bulk of the fermenting liquid over smaller vessels of cask shape, and collecting, by suitable arrangements, the yeast which flows away from an upper aperture corresponding to the bunghole of a cask. The latter method, in its various modifications, is termed 'cleansing.' The cessation of the formation of a yeasty head in skimmed fermentations, and of the flowing away (spurge) of yeast in the cleansing system, marks the end of primary fermentation.

12. Final settling and cooling down of the fermented ale either in the vessels in which the fermentation has proceeded, or in special vessels, which may hold, perhaps, the whole brewing from the different fermenting vessels, or the cleansing plant. These vessels are called settling squares or rounds, racking squares, &c. After a sojourn in the racking vessel the beer, being fairly clear and somewhat flat, is drawn off into the sale casks, the operation being termed 'racking.' Sometimes the primary fermentation is finished in the sale casks.

[1] Practically all English fermentations are carried out with 'high' yeast.

13. Where moderate storage is intended, some dry hops of good quality are generally added to each cask, the amount varying with the quality and strength of the beer. The beer then passes into the store cellar. Sometimes small quantities of sugar solution, and possibly a small quantity of some antiseptic, is added at this stage, or the addition of these may be delayed till the beer is sent out for consumption. It is necessary for the well-being of the beer that it should develop a gradual fermentation called the secondary fermentation or 'conditioning' of the beer. If this proceeds in a normal manner the beer will spontaneously brighten, a small but sufficient amount of carbonic acid gas will be formed, and the pleasant flavours looked for in a ripe beer will be produced. The behaviour of the beer in cask is perhaps the subject of more anxiety to the brewer than any other matter connected with the brewing, for here the question is answered as to the character of the materials which the brewer has used in the production of his ales, and as to the skilfulness or otherwise of the methods employed in dealing with them. If the beer is unsatisfactory, he may have the poor consolation of finding a clue to better methods for future brewings, but he cannot do much in the way of amending the defective beer.

The antiquity of beer is very considerable. It appears to have been well known to the ancient Egyptians under the name of *heqa*[1], for which the hieroglyphic or written expression was— the signs composing which are calculated to give rise to some interesting conjectures. To the later Egyptians, barley (or barley-malt) beer was known as *zythus*, and the vicinity of Pelusium was noted for the beverage. Wilkinson[2] says that as hops were unknown, recourse was had to other plants in order to give the beer a grateful flavour, and the lupin, the skirret (*sium sisarum*), and the root of an Assyrian plant, were used for that purpose. The Jews probably acquired their knowledge of leaven and its uses from the Egyptians. Coming to more recent times, we know of the *öl* (our modern 'ale') of the Vikings, and the *beor* (our modern beer) of the Saxons, and it is related in historical writings how, in more modern times still, the monks in the vicinity of Burton-on-Trent discovered the excellent brewing qualities of the waters of the district, and brewed conventual ales with Burton water

[1] *Malt and Malting*, H. Stopes, p. 4.
[2] *The Ancient Egyptians*, vol. i, p. 54.

at the close of the thirteenth century[1]; and Burton beers are not altogether unknown at the present day.

Before the act of 1880, which included the repeal of the Malt Tax and gave brewers much latitude in the employment of substitutes for malt, beer might well have been defined as a beverage brewed from malt and hops, barley being the malted cereal. Not a few people would be pleased if the definition still held good, and that the sanction given to brewers had not been of such a wide nature as to permit of beer being brewed with comparatively little malt entering into its composition. The state of things at present is such that a definition of beer is by no means easy in any simple terms, and it is left somewhat to the public taste to decide what is and what is not beer, and the public taste very often decides in favour of a beverage which is not wholly from malt and hops, when critically compared with one which is brewed wholly from the orthodox ingredients. The case as it stands bears rather a striking analogy to the economy practised in wine production known as Petiot's process; the product is not wholly and strictly the fermented juice of the grape, which genuine wine should be, and yet it may be very tolerable wine.

We will now pass to a more extended description of the brewing process, of which we have as yet but given a bare outline.

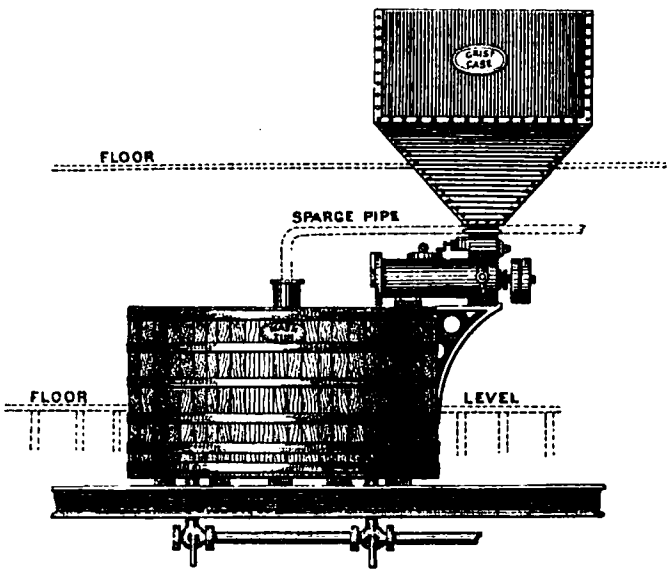
Since whole, uncrushed malt will, by reason of the impervious nature of the skin or husk, yield little or nothing to either hot or cold water, crushing is obviously necessary, and this is done very effectively by passing it at a regular rate through the steel rollers ('rolls') of the malt-mill, which thoroughly break the malt without pulverizing it. The operation is attended by the production of some little heat from the friction, and this is shown by the grist in a definite accession of temperature. It is desirable that each grinding of malt should remain for the same definite period before it is mashed, so as to let the grist come to about the same mean temperature, otherwise there will be a known source of irregularity in the temperature of the mash. It is unusual and not desirable to mash immediately after grinding. The degree of fineness or coarseness of the grist depends rather on the choice of the brewer. A medium state between coarse and fine is best, for if too coarse there will be a failure to obtain the full extract, and if too fine difficulty of drainage results, or too much may be extracted from the malt. The grist should be uniformly ground, and

[1] *Burton-on-Trent: its History*, &c., William Molyneux, p. 219.

all the corns crushed. As the grist-hopper must command the mash-tun, the position of the malt-mill generally renders it necessary to mechanically convey the grist to the grist-hopper: this is effected by means of an endless band with metal cups or pockets attached to it; the apparatus (called a 'Jacob's ladder'), acting like a dredger, picks up the ground malt from the mill and carries it upwards to the hopper. The malt should have arrived at the brewhouse reasonably free from stones, nails, string, &c., for if not, it leaves the screens of the malt-mill to do more than they are perhaps capable of, and a spark struck at the 'rolls' by a stone may ignite the whole column of flour dust in the Jacob's ladder and in the empty space of the grist-hopper, and a very serious explosion may ensue which is always alarming and may be very destructive. Malt-mills are now designed so that the dust from them does not escape so much as it used to do, and thus a source of dirt and infection is obviated. The grist-hopper should be in a dry place, well protected from steam and moist air (for ground malt absorbs moisture very rapidly from the air, and it is most undesirable that it should do so), fitted with well-closing doors or manholes, and having a good angle to facilitate the fall of the malt when mashing.

The water, or hot liquor, is now brought to the desired temperature in the liquor copper or tank. This is generally arrived at by boiling a certain volume of water and then running in cold water; or the whole volume may be heated up to the mashing temperature, but this is not to be recommended, as it is of direct use to boil the bulk of the water; moreover, it is customary and desirable prior to mashing to run enough boiling water into the mash-tun to just cover the strainer plates or false bottom. Things are now in train for mashing. The hot liquor, at a temperature of 165°–170° F., is run together with the grist into the mash-tun (Fig. 14), and water and malt are thoroughly incorporated by passing through a piece of external machinery—Steele's mashing machine is the favourite appliance, and is the one shown in the illustration—with which both water supply and grist-case are in communication, the former by a pipe with controlling tap and thermometer, the latter by a screw slide, the handle of which is conveniently situated for the grasp of the brewer, who has to adjust the flow of grist and liquor so as to keep some degree of uniformity in the ratio of the one to the other right through the operation, until all the grist has fallen from the hopper, and the predetermined amount of water has gone into the mash-tun with the grist. An average-sized mash would be about 20 quarters (160 bushels, at 42 lb. per

bushel), and an average amount of liquor of about two barrels (72 gallons) per quarter. It is better if the mash-tun be nearly filled, for small mashes in large tuns lose heat quickly, and it is necessary that the fall of temperature during the 'standing on' of the mash should not exceed a few degrees. Such a mash as we have described would have a temperature of about 152° F. in the mash-tun, and this temperature is called the 'initial heat' (the heat of the hot liquor is termed the 'striking heat'), and it is an important temperature to be taken



MASHING PLANT.

FIG. 14.

and noted down by the brewer, for it means practically the transformation temperature for the starch of the malt. In taking this temperature, it is well to let twenty minutes pass after the mash is all in, and then use a thermometer at perhaps four different points in the mash-tun, taking the average of the indications as the true initial temperature. In a well-protected mash-tun of from 20 quarters upwards a slight rise of temperature not unfrequently takes place from the heat liberated by the hydration of the starch of the malt; this is succeeded by a fall, and so after the usual time of standing on—generally about two hours—

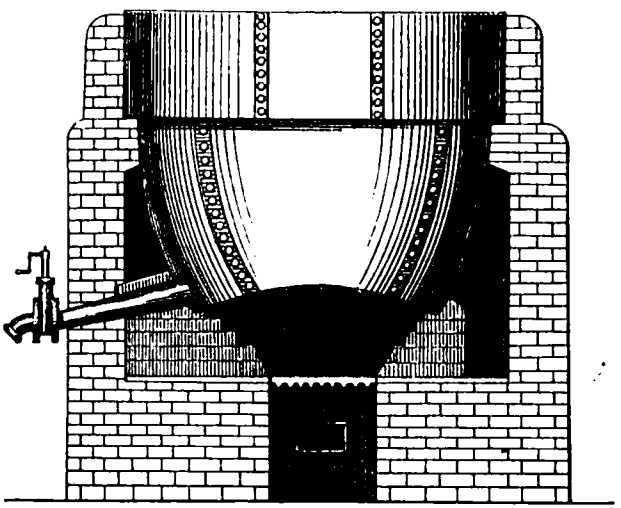
the temperature of the mash will be near 150° F., and this will be the temperature of the wort associated with the 'goods.' Changes apparent to the eye take place during the time of standing on: the original pasty, viscid mass thins out in a marked way until the husky portions can be much more clearly discerned, and the state of porridge becomes one of semi-solution, the mash growing sweeter and sweeter as the starch is transformed by the diastase, and the specific rotary power of the wort falling a great deal. At the end of two hours nearly all the starch should have been transformed, and the soluble albuminoids have been dissolved, and a clear pale or dark sherry-coloured wort, according to the kind of malt employed, should flow freely from the goods when the taps are set for running off. It is undesirable to run off the wort fast, especially at first, as it tends to drag down the goods too forcibly on to the drainage plates, and a difficulty may result from blocking of the holes or slots.

Sometimes a modification is introduced into the mashing process by using a smaller quantity of hot liquor to get the grist into the mash-tun, and then after standing for half an hour or an hour, some hot liquor is passed into the mash-tun by a pipe running into the tun under the false bottom: this serves to clear the apertures of the plates, but the more immediate object is to permit the greater part of the starch transformation to go on at a low temperature, and then to raise the temperature of the whole mash so as to restrict the action of the diastase in the further period of the standing of the mash.

The description of the passage of the sweet wort from the mash-tun through the underback calls for no particular enlargement, except to say that sweet wort should never hang about on a falling temperature. If some of it must be held back, the underback, or vessel containing it, should have a steam coil in it, by which the temperature can be maintained at not less than that at which it came from the mash-tun, or preferably higher, until the wort goes into the wort copper.

When a few or more barrels of sweet wort have run from the mash-tun, it is desirable to start sparging with hot liquor at a determined temperature, which is generally between 160° F. and 170° F. The sparger is a perforated pipe, so pivoted and balanced that the reaction of the liquor issuing in jets from the perforations causes it to rotate on a central axis. The arms of the sparger thus sweep round and round over the goods and deliver the sparge liquor in a uniformly searching stream, which effectually washes out the saccharine extract from the goods. The sparger should deliver hot liquor at

about the same rate as wort is being withdrawn from the taps. This operation may be continuous until the whole of the grains are washed clear of extract, and is usually continuous where the whole of the sweet wort is gathered into one or two wort coppers; but if the copper space does not suffice for the whole charge, the sparging is intermitted, the taps stopped, and the partially extracted goods stand at rest till the copper is vacant again. The liquid that finally comes from the goods is termed 'last runnings,' and should show a very low specific gravity, say 1005, and not give any starch reaction with iodine solution, thus evidencing the complete extraction of the malt. Last



WORT COPPER.

FIG. 15.

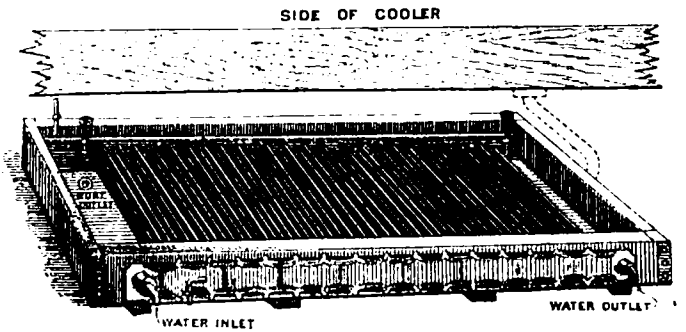
runnings are a slightly opalescent, colourless liquid, and the solids they contain consist mainly of such substances as are difficultly soluble in hot water. In some breweries the sparge temperatures are so adjusted that the 'taps' show a temperature deviating but little from the original tap-heat, say 150°–152° F. In other breweries, however, higher sparging temperatures are employed, and the indicated thermometer heat at the taps may rise gradually to 160° F., or even higher.

The boiling of the worts is the next feature of the process requiring our attention. Directly the wort copper (Fig. 15) contains an appre-

ciable amount of wort which has been either pumped to the copper or run directly into it, the copper hops are emptied out of the bags into the sweet wort, and either the fire is made up underneath the copper or high-pressure steam is turned on, according to the method of heating employed: in either case the object is to bring the wort to a state of boiling as soon as possible after the copper is charged. If the capacity of the wort copper is insufficient to take the whole brewing, the stronger first wort is termed a first copper wort, and the following one a second copper wort, and the specific gravity of the collected worts in the fermenting vessel is adjusted on the second copper. Where the whole brewing is in one copper, the final strength in the fermenting vessel must obviously be adjusted on the final volume in the wort copper after boiling. The brewer is able to bring the strength pretty near what is required by calculating the relationship of malt to water in the mash and the amount of water sparged, and it is within his power to divide the sweet wort as he pleases between different coppers, so as to produce different kinds of ale, or he can mix first and second copper worts in the fermenting vessel in any proportions he pleases. Apart from the mixing, however, there is—as indicated—a means of final adjustment by the volume known to be in the copper, the specific gravity of the wort being also known. A very usual period of boiling is from one and a half to two hours: the stronger worts may very well be boiled somewhat longer than weaker ones. The boiling must be thorough, whether done by direct fire-heat or steam. The amount lost by evaporation from the boiling wort varies not a little, according to the proportions of the vessel and the character of the ebullition; about one-eighth to one-fifth of the volume goes away in steam, and there is a corresponding increase in the strength or specific gravity of the wort, and a very decided change in the flavour, for the somewhat mawkish sweetness of the wort gives way to a pleasant, aromatic, well-cooked flavour, markedly bitter, of course, from the hops, but carrying flavours from this latter source which much enhance the general effect on the palate. There is a general impression that the well-cooked flavour is obtained with more certainty in fire-heated coppers than steam-heated ones: what difference there is, is probably due to the fact that the wort has successively passed over a more highly-heated surface in the former case than in the latter.

When the whole copperful of wort has been boiled for the desired length of time, it is ready to be 'turned out' into the hop-back. The furnace doors are opened and some of the fire removed, or the steam

is turned off and a large sluice tap is opened, through which wort and spent hops stream to the hop-back: directly the copper is out, some hot water is run in as the sluice tap is closed to prevent the bottom of the copper being burnt by the fire, the remainder of which can be withdrawn at leisure. A few minutes' standing in the hop-back allows the hops to settle on the strainer plates, and then the wort is allowed to come away from the hops, which it does readily, and is usually pumped by gunmetal wort-pumps to the wort receiver or 'cooler.' As the wort discharges into this vessel it should appear moderately bright, having left the hop leaves and seeds, and a good quantity of small extraneous matters in the hop-back. The scalding wort on the cooler soon falls some 20° or 30° F., and cools more slowly after this; at the same time it deposits a certain amount of buff-

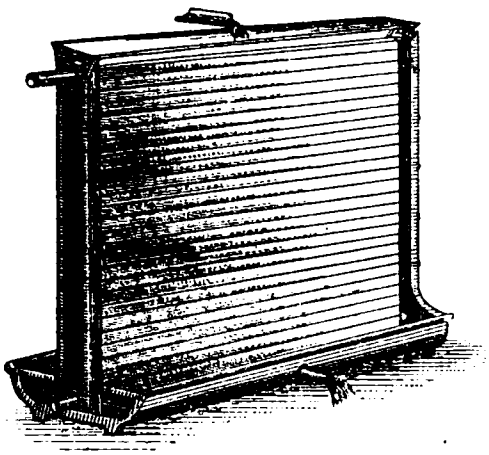


HORIZONTAL REFRIGERATOR.

FIG. 16.

coloured sediment called 'cooler grounds,' and the partially cooled wort is run off towards the refrigerator in a gradual manner so as to leave this sediment behind. [The 'grounds' are generally gathered afterwards by a 'squegee' and filtered through felt bags to obtain the wort that is with them.] From the cooler the wort runs by gravitation to the refrigerator (Figs. 16 and 17), and it is desirable that the whole cooling operation should be conducted as speedily as the appliances will permit. It will be readily understood that it is by no means easy to get the worts cooled quickly in the warmer months, for the temperature of the cooling water is probably higher, and perhaps a high atmospheric temperature has to be contended with. In the summer and autumn too the risk of aerial infection is greatly increased, and

may have a marked effect on the stability of the beer. The longer the worts remain exposed on the cooler, the greater will be the degree of infection by floating organisms, microbes, &c.: it is therefore greatly to be recommended that the refrigerator should be more powerful than is needed, rather than less so. At the best it takes two or three hours to get an ordinary copperful of wort cooled and refrigerated and collected in the fermenting vessel.

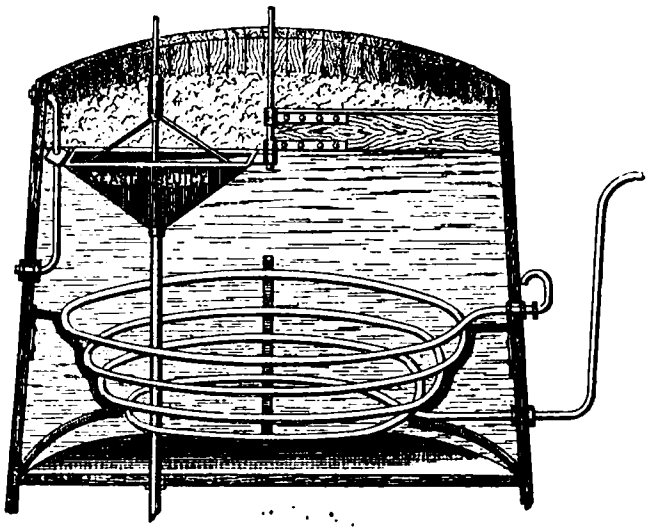


VERTICAL REFRIGERATOR.

FIG. 17.

From the refrigerator, the cooled wort—now somewhat turbid by the separation in a fine state of division of certain matters thrown out at the lower temperature—runs into the fermenting vessels (Fig. 18) at a pre-determined temperature, and directly a few barrels have been gathered the pitching yeast is added at an average rate of about 1 lb. per barrel for mild ales, 1½ lb. to 2 lb. for pale ales, and 2 lb. and upwards for strong ales. The collection of the worts goes on till each fermenting vessel (called 'squares' or 'rounds' according to their shape) has received its full charge of wort. The temperature of collection averages from 58°-60° F., and for the next eight or ten hours the wort remains practically quiescent, the temperature not falling back and not rising. After this, however, visible signs of fermentation are apparent, a white creamy froth appears on the surface, which develops into a white curly head, and the temperature begins to rise and the specific gravity of the wort to decrease: the curly head becomes a white rocky head, showing perhaps some 'grounds,' and the head then becomes more frothy with larger gas bubbles, till it at length stands possibly 3 feet above the level of the liquid, being confined by the upper margin of the square or round. The tempera-

ture now rises more and more rapidly, the gas bubbles of the froth tend to break more readily, and then the head begins to give way and sink, and concurrently with this there are indications of the presence of yeast in the frothy head, and the temperature of the whole bulk is either near the maximum heat attained—usually quite 10° F. above the pitching temperature—or has been at the maximum, and is being prevented from rising higher by running cold water through cooling pipes—known as attemperators—which are a part of the arrangements of the fermenting vessel.



FERMENTING VESSEL WITH ATTEMPERATOR AND YEAST SLUICE.

FIG. 18.

The working maximum of a brewery fermentation is commonly attained when the specific gravity of the fermenting wort has fallen to somewhere about one-half the original specific gravity, and this is rather an important stage in the operation, for it is at this point that the question as to the mode of treatment to be adopted must modify the further description of the process. In other words, the procedure up to the stage just mentioned is fairly similar for either the skimming or the cleansing process, only that in the latter case attemperators are not often used in the fermenting vessel,

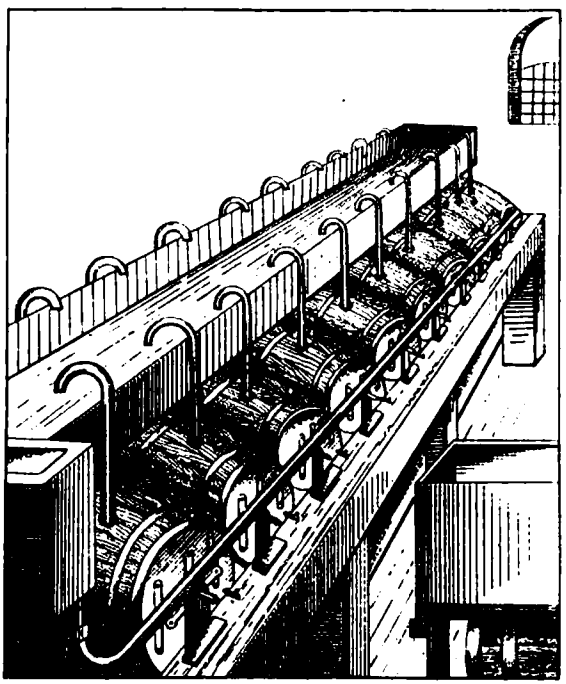
whereas in the other case the attemperators would have been set on gently some few degrees lower than the maximum temperature attained, and an early check given to the rate of fermentation.

Taking into consideration a plain-skimmed fermentation pitched at 60° F., we may repeat that slow control by the attemperators would be instituted at about 67° F., or from thirty-five to forty hours from the time of pitching: a not very clean-looking yeasty head would perhaps be skimmed off and rejected, the next following crops of clean yeast would be gathered carefully, and retained in suitable yeast vessels or troughs, with the idea of their being available for setting or pitching subsequent brewings. The further development of a fermentation, such as we are describing, is that the loss of specific gravity and the yield of yeast run—or should run—*pari passu*, till from want of fermentable matter fermentation slackens and fails, the temperature of the beer coming slowly down to about 55°-60° F. again. If the fermentation has been carried right through in the same vessel, a good deal of yeast, largely the older cells and grounds, is found on the bottom of the square or round. Sometimes the beer is, after a period of settling, racked straight from the fermenting vessels into the sale casks, or it goes to a racking square.

A modification of the skimming system which gives very excellent results is to run the half-fermented wort into shallower wooden vessels —usually rectangular in shape—called 'dropping squares'; in this case the grounds are left behind in the fermenting vessel, the beer receives a somewhat salutary accession of oxygen from the air during its transit to the succeeding vessel, and the shallower layer of fermenting liquid favours the throwing up of full and healthy crops of yeast—other conditions being favourable. Fermentation is practically completed in the 'dropping squares,' and the beers may be racked from these, or more conveniently from a racking vessel. There are various methods of skimming and appliances for performing the same, but it would be going beyond our mark to enter into details which are better grappled with in the brewery itself.

The cleansing system in its primitive form consists in running the partially fermented beer into a cask, and letting it work itself out through the bunghole, filling up the space at intervals with some fresh fermenting wort or beer till the primary fermentation is over and the excess of yeast has been expelled, some remaining yeast being found at the bottom of the cask. The first important modification of this system was to let the fermenting wort from the original fermenting

vessel run, at the stage we have already spoken of, into large casks or 'tunners' holding four or five barrels apiece. These were mounted on a trough, and the yeast flowing out of the bunghole and down the sides gathered in the trough: this rather dirty method was improved upon to the extent of putting discharge pipes into the bungholes, and the 'tunners' being on a thrall or stillion about 2 feet high, the pipes

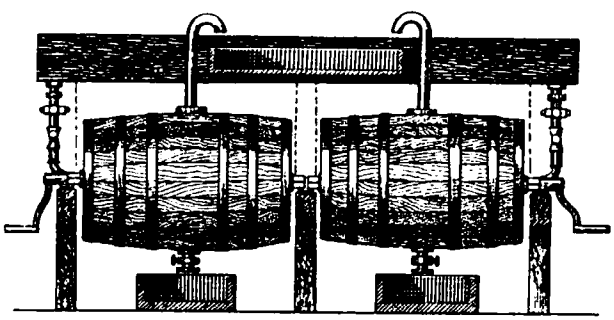


DOUBLE SET OF UNIONS.

FIG. 19.

discharged the yeasty beer and yeast into troughs set underneath the points of discharge; a separation of yeast from yeasty ale (barm ale) took place in the troughs, and the barm ale was used at regular intervals for filling up the vacancies in the 'tunners' caused by the spurge. The beer was ultimately racked from the 'tunners.' A very much better system, and one much used in Burton, is the modification of the above method, known as the Burton union system. The essential

features of it are these: The cleansing casks are arranged in double sets, an equal number of union casks being on each side of a central trough (Figs. 19 and 20). Each union cask has an attachable pipe (swan neck) bending over into the trough. At right angles to the central trough, communicating with it, and at a lower level than it, is another trough called the feed trough; this receives the barm ale running from the yeast in the centre trough, and it is connected by pipes running the whole length of the union casks with each and every cask on each half of the set. The feed trough can therefore be used, and is used, for filling the union casks with partially fermented beer in the first instance. The casks are filled till frothy beer runs from the swan necks into the long trough: they are then left to spurge just as cleansing casks do, and filled up at regular intervals from the barm ale in the

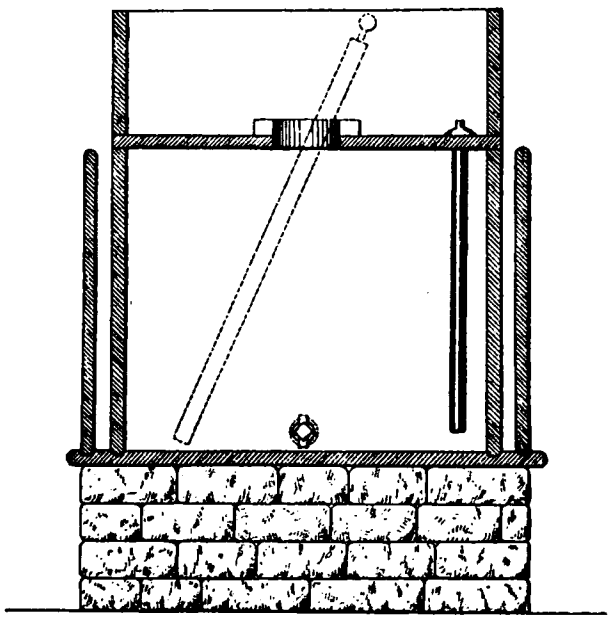


END VIEW OF UNION SET.

FIG. 20.

feed trough till fermentation is finished and no more yeast comes from the swan necks, even with the union casks full of beer. In the summer months attemperating pipes for the circulation of cold water are used in the union casks and in the yeast troughs. Unions are set up with axes in trunnions, so that when the side pipes are disconnected and the holding bolts released, they can be rapidly rotated by fitting a handle on to the squared end of the front axis and turning. Scalding water is run in and discharged by bringing the discharge tap—which is immediately and vertically under the socket into which the swan neck fits—into its normal position, and running off; or the cleansing water can be run off from the 'bush' or socket of the swan neck. It is from the lower tap that the fully fermented beer has been previously run to

a racking square in which the final settling takes place, and the beer falls a few degrees further in temperature, coming to 60° F. or a little lower. The total sojourn in the unions is about four days. The time occupied in a complete brewing on practically any system is seven days. Where beers are passed at the close of the skimming or the cleansing to a racking vessel they should be nearly flat. It is advantageous, however, if they throw up a very thin film of yeast, as it prevents over-flattening from too complete escape of the



"STONE SQUARE" FERMENTING VESSEL.

FIG. 21.

carbonic acid gas, and also prevents the escape of aromatic principles. A long stay in the racking vessel is not desirable, twelve hours is usually ample. From the racking vessel the beers, which should be fairly bright, are drawn from taps into the sale casks.

The casks into which the beer is racked must be scrupulously clean and dry. Cask plant requires great care in its management, for it can be readily realized that apart from the ordinary wear and tear of a somewhat perishable receptacle, the treatment to which casks are

subjected in the trade—such as leaving them exposed to air and rain with grounds or lees souring and mouldering in them—is not calculated to help the brewer to turn out a delicately flavoured beer. A cask once badly infected by mould is practically incurable, and is only fit for storing paraffin, rain-water, &c., or for firewood.

A few words about a method of fermentation which partakes somewhat of the nature of both skimming and cleansing. This is the Yorkshire stone square system, an antique and interesting process. The fermenting vessels in this case, instead of being of wood, copper-lined wood, or copper, are built up of slabs of stone or slate carefully cemented together (Fig. 21). The covering slab has a circular orifice some 18 inches in diameter, and surrounding this is a raised ring or flange some 6 inches in height; the sides of the vessel rise a full 2 feet above the level of the covering piece, and constitute a receiver or yeast trough. The vessel has two other narrow apertures, one of which is provided with a valve or trap which allows liquid to run down from the upper receptacle by means of a pipe to the bottom of the stone square, the other is an aperture fitted with a movable plug, into which a pump, also going to the bottom of the square, can be introduced or withdrawn. The whole of the area of the sides of the vessel, up to within a few inches of the level of the covering piece, is duplicated or jacketed by a surrounding water-tight vessel or arrangement of slabs, a few inches intervening between the two surfaces. Cold or warm water may be kept or circulated within the jacket, which thus plays the part of an attemperator, and can be used to either accelerate or retard the fermentation going on in the stone square. The worts having been gathered within the vessel, which is filled nearly up to the manhole, the rising head discharges through the latter and tends to fill up the receiver; the fermenting wort, controlled by attemperating, is also frequently aerated by a definite number of strokes of the pump at regular intervals, which results in the beer being pumped into the receiver, and then falling back into the square through the small valve hole. As fermentation proceeds, and the head becomes yeasty, the flange causes the yeast to be retained in the upper part, whilst the barm ale is allowed to flow back into the square. The end result is that practically all the thick creamy yeast is in the top receiver, whilst the quiescent fermented ale is undergoing its final settlement in the stone square prior to racking. Very nice smooth ales are produced by this method. The yeasts are somewhat slow in their action, but sure. The cells

generally show the peculiarity of well-marked spherules in the vacuole. The temperatures shown throughout the progress of a stone-square fermentation would not be very dissimilar to those of a slow-skimming fermentation. The pumping-over, and the way in which the yeast is caused to separate, are the salient features of this particular method, which—owing to the expense of the vessels, and some disadvantages, such as the extra supervision required, and the increased difficulty of keeping the vessels sound and sweet—is not at all in general use.

Practical considerations connected with the treatment of beers in store or cellar, and such matters as bottling beers, important though they are to the brewer, cannot be adequately dealt with in these pages. We must refer the reader who seeks more information to the standard works on the technology of brewing. The best place to learn the practice of brewing—if not the science—is in a brewery, and after all is said and done, the technical operations can only be properly learnt there. We have merely aimed at giving such an outline of the process as would render the scientific considerations in relation to it more intelligible.

The Scientific Aspect of the Brewing Process.

We may now pass on to the chemical and physical changes taking place at the various stages of the brewing process: matters which are not self-evident to the brewery pupil, nor discernible by the ordinary use of the powers of observation. Matters too which are not within the ken of every brewer, highly skilled though he may be in the general technology of his process, but which it behoves every brewer, so far as he is able, to make himself acquainted with. We will take the subjects in the order in which they come in the brewing process:—

The Brewing-Water, or Liquor used for Mashing and Sparging.

Were the extraction of the soluble matters of the malt the only object, it would not, within certain limits, matter very much what kind of water was employed for mashing and sparging if it were clean and wholesome. There are, however, definite effects produced on the composition of the worts, traceable to the particular saline substances which the water holds in solution, and every brewer should have at least some general idea of the effects ascribable to one and the

other of the constituent salts, and whether these effects are of a nature favourable to the process or otherwise. The chief directions in which the saline substances take effect are:—

1. A modifying influence on the action of the diastase, leading to variations in the proportions of maltose, dextrin, and malto-dextrin derived from the starch of the malt.

2. Action on the nitrogenous constituents as regards the quantity and quality of the matter brought into solution, and especially as regards its suitability or otherwise for yeast nutriment.

3. The action of the mineral matters as regards the inorganic nutriment of yeast.

4. The action of the salts as regards the extraction of the soluble constituents of the hop.

5. Considerations respecting colour and flavour of beers. Power of ready-fining or conditioning, and stability.

Subordinate to the above considerations is the simple question as to the amount of extract obtained from the malt by waters of essentially different characteristics.

These influences react on the fermentations each in its own particular way: (1) has to do with the question as to whether the worts are of a readily fermentable character or otherwise, also as to whether the secondary fermentation is likely to be too pronounced, normal, or too sluggish [it should be remembered that we are speaking of the action of the saline substances as apart from variation in the temperature of the mash, which must be taken on its own merits].

Influences (2) and (3) react directly on the yeast, whose fermentative power is modified favourably or unfavourably according to the presence or absence of the suitable alimentary substances. Not only is the yeast affected as regards the fermentation it is carrying on in a given wort, but its behaviour as pitching yeast in subsequent fermentations, depends a good deal on the kind and quantity of the aliment it has received in the preceding worts. Influences (4) and (5) will be dealt with presently.

We may conveniently classify the different kinds of water used for brewing as follows:—

Class I. Very soft water, containing only a few grains per gallon of saline matters in solution. This is practically pure upland rain-water, and as examples we may mention Loch Katrine (Glasgow supply), Thirlmere (Manchester supply), Lake Vyrnwy (Liverpool supply). (Example 1, Table.)

Such waters as these are in their native condition only suitable for brewing black beers, for which a highly extractive water is advantageous. Waters of this class which are not distinctly alkaline can be hardened artificially so as to fit them for ale-brewing.

Class II. Calcareous waters from chalk formations, limestone, &c., the solids of which consist chiefly of carbonates of lime and magnesia (carbonate of lime usually predominating).

A. Moderately hard, containing about 20 grains per gallon of solids, mainly carbonate of lime. (Example 2.)

B. Hard, containing about 40 grains per gallon of solids, largely carbonate of lime. (Example 4.)

These waters throw out most of their solids on boiling, and though sometimes used alone, are now generally hardened artificially for brewing.

The hardness removed by boiling is known as 'temporary hardness.'

Class III. Sulphated or gypsum waters.

A. Hard, such as those from the valley gravels of Burton-on-Trent. (Example 5.)

B. Very hard, such as those from artesian bores in the Keuper marls and water-stones of Burton-on-Trent. (Example 6.)

These are the waters so suitable for pale and stock-ale brewing, and which have contributed so largely to build up the reputation of Burton ales.

Class IV. Alkaline waters containing sodium carbonate, &c., such as those from the green-sand underlying the London clay, and similar formations. (Example 7.) The waters are excellent for stout and porter brewing, but are useless for pale ales.

There are well-marked instances of waters whose composition lies perhaps between two classes, or partakes of the characteristics of different classes such as II and IV. (Example 8.)

WATER ANALYSES, EXPRESSED IN GRAINS PER GALLON.

	1	2	3	4	5	6	7	8
	Class I. Very soft.	Class II. A. Calcareous.	Intermediate. Calcareous.	Class II. B[1]. Calcareous.	Class III. A. Hard. Gypsum.	Class III. B. Very hard. Gypsum.	Class IV[2]. Alkaline.	Mixed. Alkaline and calcareous
Calcium carbonate	Small quantities of chlorides, nitrates, and sulphates, chlorides predominating.	11·2	14·00	21·34	14·0	14·0	5·37	14·78
Magnesium carbonate		1·5	1·05	1·03	3·5	2·1	3·82	5·25
Sodium carbonate		—	—	—	—	—	8·25	9·48
Calcium sulphate		1·4	2·67	8·74	31·5	102·9	—	—
Magnesium sulphate		—	—	—	11·9	22·4	—	3·83
Sodium sulphate		—	—	—	—	0·7	16·65	12·67
Calcium nitrate		1·6	2·94	5·33	—	—	—	—
Magnesium nitrate		—	—	—	6·3	2·8	—	—
Sodium nitrate		1·2	1·57	—	—	0·7	0·69	—
Calcium chloride		3·5	—	—	—	—	—	—
Magnesium chloride		—	—	—	1·4	0·7	—	—
Sodium chloride (and a very little potassium chloride in some cases)		—	5·88	3·28	8·4	7·0	8·54	13·26
Silica, iron, alumina, &c.		0·5	0·76	1·14	0·7	0·7	0·39	0·93
Total solids	3·15	20·9	28·87	40·86	77·7	154·0	43·71	60·20

[1] Morris and Moritz, *Text-book of the Science of Brewing*, p. 4.
[2] Ibid. p. 7.

[The remainder are either from F. E. Lott's or from Matthews and Lott's records.]

Speaking generally, the softer waters, in anything approaching to their natural state, are used in conjunction with mashing temperatures which give a rather more fermentable wort, and one which from the nature of the water contains nitrogenous matter in solution in a state not so readily assimilable by yeast. These facts necessitate a slow kind of fermentation—usually a skimming or stone-square method—inaugurated and carried on by a yeast which is relatively of a somewhat slow habit. Rousing is a common resource in such a process, the fermenting worts being mechanically roused from time to time in the fermenting vessels, and by means of the pump in the stone-square system. A lower specific gravity—or 'attenuation,' as it is called—is also reached than if hard waters of the Burton type had been employed, four-fifths of the specific gravity as indicated by the saccharometer being usually fermented away; that is to say, a wort of specific gravity 1055 shows at the end of the primary fermentation about 1011. Beers produced on these lines taste full and pleasant; but unless much care and skill are exercised they are very prone to develop a too vigorous secondary fermentation, with cloudiness from the formation of new yeast—generally of a species of Saccharomyces other than S. Cercvisiae or the normal pitching yeast. This abnormal secondary fermentation constitutes a 'fret,' and has its origin mainly in three causes.

1. The presence of easily reducible or degradable malto-dextrin or amylöin matter.

2. An excess of nitrogenous yeast-feeding matter.

3. An excess of phosphates, especially potassium phosphate.

Frets are more likely to occur in the warmer months, as the enhanced temperature favours fermentation, and the pitching yeast is not so active as in the winter months. The best general remedy is to take steps to secure as vigorous a fermentation as possible, and a lower attenuation.

The hard sulphated waters of the Burton type are for the most part used in conjunction with rather higher mashing and sparging heats, with a fast kind of fermentation and cleansing in tunners or unions. Skimming methods are sometimes employed, in which case the fermentations are controlled, if desired, by attemperating. There is no doubt that a strong vigorous yeast is produced by the Burton methods which does its work rapidly and effectively. Burton worts are on the average attenuated to about one-fourth the original specific gravity. The malto-dextrins, being of a less easily reducible character,

are fermented slowly by the kinds of Saccharomyces taking part in secondary fermentation, whilst the nitrogenous matters of the worts, being of a more assimilable nature, are largely removed as yeast crop. The phosphates too are removed to a very considerable extent, as calcium phosphate in the cooler grounds, &c. These facts make for a stability of beers brewed with hard gypseous waters—apart from other advantageous circumstances of colour, flavour, &c.—which is unattainable with softer untreated waters.

The examples given of different water supplies suffice to show that great diversity may exist in brewing-waters in different parts of the kingdom. The position is, however, simplified in some measure by the fact that there is a general tendency at the present time to bring all brewing-waters by suitable treatment somewhat into line with the Burton supplies. It will be profitable, therefore, in discussing the action of the chief saline substances in water, from the brewing point of view, to direct our attention more particularly to the salts that figure in the Burton waters. We will take the substances *seriatim*, saying what we can about each :—

Calcium Carbonate ($CaCO_3$). Held in solution in the water by carbonic acid gas. Mainly removed from that portion of the brewing-water which is actually boiled. The larger or smaller quantity left, and that which comes in with the rest of the brewing liquor, is believed to react as follows :—

1. Partially neutralizes the free acid of the malt (due to acid phosphate of potash) giving secondary and tertiary phosphates.

Primary phosphate Dihyd. potass. phosphate		Calcium carbonate		Hyd. monocalcic phosphate		Hyd. potass. carbonate
KH_2PO_4	+	$CaCO_3$	=	$CaHPO_4$	+	$KHCO_3$
Secondary phosphate						
K_2HPO_4	+	$CaCO_3$	=	$CaHPO_4$	+	K_2CO_3
				Calc. triphosph.		
$CaHPO_4$	+	$CaCO_3$	=	$Ca_3(PO_4)_2$	+	$CO_2 + H_2O$.

Also

$$3CaHPO_4 + 2K_2CO_3 = Ca_3(PO_4)_2 + K_2HPO_4 + K_2CO_3 + CO_2 + H_2O.$$

The calcium triphosphate formed is thrown out as an insoluble substance during the brewing process.

Calcium carbonate, when present in considerable quantity, tends to impede the action of diastase, also to give colour to the worts and beers by extracting colouring matter from the malt, and by darkening the colour of matters extracted from the hops. It also tends to

turbidity of worts and difficulty of brightening of beers, and somewhat impairs the flavour. These remarks apply only to cases where the carbonate of lime exceeds 10 or 12 grains per gallon, and constitutes the chief constituent of the brewing-water; in the latter case well boiling the liquor is especially to be desired prior to mashing.

Magnesium Carbonate ($MgCO_3$). Held in solution in the water by carbonic acid gas. Not readily removed by boiling the water. Seldom present in marked quantity. Should it be so, however, the following effects may be looked for—partial neutralization of the acid phosphates of the malt wort leading to the formation of magnesium phosphate thus:—

Hydric magnesium phosph.

$$2KH_2PO_4 + 2MgCO_3 = 2MgHPO_4 + K_2CO_3 + CO_2 + H_2O$$

$$K_2HPO_4 + MgCO_3 = MgHPO_4 + K_2CO_3.$$

Magnesium phosphate being somewhat soluble in malt worts is not thrown out readily during the brewing process.

Magnesium carbonate reacts somewhat similarly to calcium carbonate; it somewhat impedes diastatic action, increases the colour of the worts, and is said to increase the extract from the malt. Magnesium carbonate is likely to have the more marked effect when carbonates of lime and magnesia are the main constituents of the brewing-water.

Sodium and Potassium Carbonates. The former is uncommon and the latter substance rare; they may be taken together for our purpose, as their action is rather similar. The effect produced by alkaline carbonates may chiefly be considered in connexion with truly alkaline waters, for when found—and this is not common—in conjunction with calcium and magnesium salts, chemical reaction sets in directly such a water is raised to boiling, leading to the precipitation of a quantity of lime and magnesia as carbonate equivalent to the alkaline carbonate present; thus, for instance:—

Calcium sulphate		Sodium carbonate		Calcium carbonate		Sodium sulphate
$CaSO_4$	+	Na_2CO_3	=	$CaCO_3$	+	Na_2SO_4
Magnesium sulphate		**Sodium carbonate**		**Magnesium carbonate**		**Sodium sulphate**
$MgSO_4$	+	Na_2CO_3	=	$MgCO_3$	+	Na_2SO_4.

Alkaline carbonates impede diastatic action very markedly, and they heighten the colour of the worts by dissolving out coloured matters from both malt and hops. A coarse-flavoured beer is produced by the solution of a larger amount of resins, &c., from the hop. A larger

quantity of the difficultly soluble albuminoids of the nature of globulin, &c., is brought into solution, and this much interferes with clarification of the beers. Alkaline carbonates act advantageously for black beers, stouts, &c., as the albuminoid matter dissolved increases the 'body' or palate fullness of the resulting beverage, and the colouring-matter is more fully extracted from the amber and black malts used for porter-brewing.

Calcium Sulphate ($CaSO_4$). **Natural Gypsum** ($CaSO_4$, $2OH_2$). If present in moderate quantity, say up to 30 grains per gallon, it does not diminish extract; but when present in large quantity, say over 100 grains per gallon, it appears to perceptibly diminish the extract obtained. Calcium sulphate waters—or gypseous waters as they are sometimes called—possess, when the other constituents stand in a good relationship to each other and to the gypsum, properties which are very advantageous to the brewer. Such waters give pale worts and produce beers of a nice golden shade of colour. They extract enough and not too much from the hop, and so the colour of the wort is kept down, and a certain delicacy of flavour is produced quite unobtainable with the highly extractive alkaline and calcareous waters, unless they have been skilfully dealt with by addition of the substances they lack. The beers drink clean and full, they clarify readily, and possess much stability. Gypseous waters promote a healthy and vigorous fermentation as they tend to the maintenance of a definite yeast, uniform in its action, and not difficult to maintain in a satisfactory state, if due care and attention be bestowed to those points of the process which are known to ensure success. Calcium sulphate interacts—more particularly in the wort copper—with the potassium phosphates of the wort, and forms insoluble calcium phosphate and potassium sulphate thus:—

$$KH_2PO_4 + CaSO_4 = CaHPO_4 + KHSO_4$$
$$2K_2HPO_4 + 3CaSO_4 = Ca_3(PO_4)_2 + K_2SO_4 + 2KHSO_4$$

Also, subsidiary reactions are possible:—

$$KHSO_4 + CaCO_3 = CaSO_4 + KHCO_3$$
$$KHSO_4 + MgCO_3 = MgSO_4 + KHCO_3.$$

This removal of a portion of the phosphoric acid is of the first importance, as phosphates are in excess in the malt and may constitute a source of danger in the worts, as they are capable of nourishing bacteria as well as yeast. The instability of soft-water brewed beers is largely due to this cause. Potassium sulphate is a first-rate mineral

nutrient for yeasts of the Burton type—if not for the Saccharomyces generally—not only on account of its providing potassium, but from the conjunction of this base with sulphuric acid (Dr. Stern has recently shown that yeast requires sulphates for its favourable development). Calcium sulphate, owing to the elimination of phosphates which it causes, has a very marked effect in precipitating certain albuminous matters in the wort copper; the coagulum formed being weighted by the calcium phosphate, readily falls out, and exercises a fining action on the worts. This is called 'breaking,' and when it takes place thoroughly the boiling wort should, if a sample be taken out, throw down its suspended matters readily, and leave a liquid which is brilliant whilst hot, but which becomes more or less cloudy on cooling. Calcium sulphate does not appear to dissolve materially less albuminoid matter from the malt than the soft extractive waters, but there can be little doubt that the nitrogenous matters are in a form in which they are more readily assimilated by the yeast, and this more complete removal of the nitrogenous matters during the fermentation of Burton worts is a further cause of the stability of Burton beers. There appears to be some restriction of diastatic action by Burton waters, which leads to the formation of malto-dextrins, or degradable reducing dextrins, and the result of this is a steady secondary fermentation in cask, whereby the beers not only are in good condition, but the danger resulting from a state of quiescence is obviated; for if a beer is not in a state of fermentation—however slight—it is most likely to fall a prey to other organisms than Saccharomyces.

Magnesium Sulphate. Anhydrous, $MgSO_4$; Crystalline, $MgSO_4$, $7OH_2$ (Epsom salts). Usually found in waters which contain calcium sulphate, and therefore in practically all Burton waters. The remarks made about calcium sulphate hold good in some measure for magnesium sulphate. Southby considered that a good relationship of the total calcium salts in a brewing-water to the total magnesium salts was 3 : 1, and very nearly this ratio is arrived at if the average shallow well water (No. 5 in the Table) is regarded as mixed in equal proportions with the deep bore water (No. 6 in the Table). Such a mixture would answer very well for Burton brewing. Other proportions will, however, unquestionably give good results.

Magnesium sulphate has some restrictive effect on diastatic action leading to the formation of malto-dextrin. In moderate quantities it does not diminish extract. It tends to pale worts. If present in large quantities it tends to a raw cold flavour in the beers, and sometimes

a not inconsiderable aperient effect on the consumer. Magnesium sulphate interacts with the potassium phosphates forming moderately soluble magnesium phosphates thus :—

$$KH_2PO_4 + MgSO_4 = MgHPO_4 + KHSO_4$$
$$K_2HPO_4 + MgSO_4 = MgHPO_4 + K_2SO_4.$$

This magnesium phosphate is one of the chief mineral nutrients of the yeast, the phosphoric acid standing first in value, and the magnesia second (vide composition of yeast ash, p. 124); both base and acid are nutrients in this case, which is not quite so with potassium sulphate, for yeast only contains traces of sulphate; doubtless the sulphuric acid is of indirect value.

Sulphate is the best form for the magnesia to be present in a brewing-water; a small amount of magnesium chloride is not objectionable. Magnesium in the form of nitrate, if present to the extent of more than a few grains per gallon, is decidedly objectionable.

Sodium Sulphate (Na_2SO_4) is a not infrequent constituent of brewing-waters. Potassium sulphate is rare. Sodium sulphate in quantity of a few grains per gallon is favourable to fermentation, without impairing flavour, but if it be present in quantity—say over 10 grains per gallon—although it is still favourable to fermentation it communicates a cold and somewhat harsh flavour to beers. This does not seem to apply to potassium sulphate; perhaps because it is so readily assimilated by Burton yeast, and probably other types of yeast as well.

Calcium Chloride ($CaCl_2$). Sometimes a natural constituent of brewing-waters. It is of questionable value, but it is certainly preferable to have some calcium in this form rather than that the brewing-water should be deficient in calcium salts other than carbonate. Calcium chloride tends to restrict the action of diastase giving slowly fermentable malto-dextrins. It tends to diminish yeast crop, and gives a yeast which does not press well and becomes sticky. Briant says it increases the total nitrogenous matters in wort, and since it tends to increased palate fullness it probably dissolves some of the globulins and other of the less readily soluble albuminoids. Calcium chloride is better adapted for the production of mild than pale ales, for although the flavour of the beer is fuller, it is also coarser, especially if the quantity of calcium chloride be at all marked.

Magnesium Chloride ($MgCl_2$). Comparatively little is known about the action of this substance. It is most probably productive of

the same results as calcium chloride, but in a more marked degree, especially as regards the tendency to impair the flavour of the beer. In quantities of a few grains per gallon it is likely to be beneficial rather than otherwise.

Sodium Chloride (NaCl). Common salt. Potassium chloride (KCl) rarely present. Sodium chloride in moderate quantity is beneficial in a brewing-water. It probably slightly enhances diastatic action. Promotes palate fullness by dissolving albuminoid matter of the nature of globulin, &c., and increases the stability of the beers. It is probably directly favourable to fermentation. Quantities up to 10 grains per gallon are consistent with the production of pale ales, but over this, and up to perhaps 20 grains per gallon, the water—other things being favourable—is more suitable for mild ales, for although palate fullness is secured, there is nevertheless some increase in colour and less delicacy of flavour.

Nitrates may be discussed together. Calcium nitrate is infrequently found. Magnesium nitrate is rather frequently found in Burton shallow well waters. Sodium nitrate is not unfrequently found. Nitrates in quantity point, of course, to an unsatisfactory origin of the water, inasmuch as they are usually derived from oxydized nitrogenous organic matters of the nature of sewage. Still, if a brewing-water contains say 5 or 6 grains per gallon of nitrates, and does not show direct contamination, it may prove a not undesirable supply for brewing purposes. The general consensus of opinion is that nitrates—especially nitrate of magnesia—act prejudicially on yeast owing, doubtless, to the fact that under certain circumstances they are reduced by the yeast, and the liberated oxides of nitrogen exercise a very prejudicial effect on the cells; the effect of nitrates seems to be cumulative, and when deterioration of the yeast from this cause has once set in, the value of the yeast as a ferment diminishes rapidly, and a very usual accompaniment is a marked increase in colour in the racking beers. It is probable that yeasts do not attack the nitrates in a fermenting wort until the supply of assimilable oxygen—free and in loose combination—is used up.

Nitrites are even more objectionable in a brewing-water than nitrates, but they are rarely present in quantity sufficient to cause any ill effect. The writer has determined by direct experiment that Burton yeast can reduce nitrates to nitrites.

Nitrates in appreciable amount tend to impair the flavour of beer. The nitrates of the alkalies are less objectionable than those of the

alkaline earths, probably because they are less readily decomposed by yeast.

The small quantities of silica, iron, and alumina existing in brewing-waters generally, may be disregarded as exercising any influence on the fermentations, &c.: they are rather useful than otherwise. An appreciable amount of iron, say over one grain per gallon, would constitute an objection—but not a fatal one—to the supply. Larger quantities of iron would be quite inadmissible, as an unhealthy tint would be communicated to the worts, and possibly to the beers and the yeast crops.

It will be easily inferred from the preceding matter that the subject of brewing-waters is not to be done justice to in a limited space. We have tried to give some of the more salient features of the subject, and have yet a few remarks to make later on. The analyses quoted in the Table, if taken in conjunction with what we have said about the action of the several saline components, will give a clue as to the kind of brewing-water which may be expected to produce satisfactory beers. For wider and fuller information we would refer the student of brewing to the large textbooks on the subject and the *Transactions of the Institute of Brewing*[1].

The Mashing Process.

We have already given some indications of the effect which the saline constituents of the brewing-water exercise upon the composition of the wort, and we need not enlarge upon the matter excepting to say that the more directly traceable influences of the hard gypseous waters may be summarized as follows :—

1. The extraction of the albuminoids of the malt in a state more favourable for yeast nourishment.
2. A modification in the action of the diastase of the malt, leading to the production from the starch of soluble carbohydrates of a less readily fermentable kind.
3. The presence in the worts of saline substances favourable to the fermentative power of the yeast and the healthy development of the cells.

The converse of these summaries holds fairly well if very soft waters be employed for brewing purposes (excepting in the case of

[1] See especially 'Brewery Waters,' F. E. Lott (*Trans. Fed. Inst. Brewing*, vol. iii, No. 5).

black beers), and intermediate results may be looked for with waters of intermediate composition. It should be remembered, however, that brewing methods can be modified—especially as regards the fermentations—to cope with the less favourable water supplies; hence the different systems in vogue in different parts of the kingdom. They are the outcome of hard-earned experience.

The next question in mashing which calls for scientific consideration is, as to the effect produced by variations in the volume of water in relation to malt mashed. If two barrels per quarter be taken as the normal—a quantity of water less than this, by giving a mixture of less specific heat (water = 1, malt = ·46), tends to increase the quantity of maltose and consequently the fermentability of the wort; whilst a volume of water above the normal, owing to the higher specific heat of the mixture, tends to restrict diastatic action, and so more dextrin (and possibly malto-dextrin) is produced and a less fermentable wort obtained. The following figures will elucidate this point:—

	Thick mash under 2 brls. per qr.	Thin mash over 2 brls. per qr.
Extract = dry solids per cent. of the malt	64·0	64·5
Total sugars in wort	81·8	75·2
Dextrin	4·8	9·9
Inactive matter, chiefly albuminoids	13·4	14·9
	100·0	100·0[1]

As regards the temperature of the mash; where underletting is practised [i. e. a low initial heat of the mash is secured, say 145° F., and at the expiration of three-quarters of an hour to one hour a quantity of water at a temperature of 165° to 170° F. is run in under the false bottom] and the diastase acts first at a lower then at a higher temperature, the net result is to give a wort which contains a fair proportion of maltose in the free state, together with a considerable percentage of not very difficultly fermentable malto-dextrins or reducing dextrins. A wort is thus obtained which ferments to a fairly low point, and the resulting beer enters with some readiness into secondary fermentation, and so 'conditions' readily. There is always a risk, however, of abnormal secondary fermentation or 'fret' with beers

[1] This was evidently a highly diastatic malt, as it yielded dextrin percentages much below the normal figure; compare p. 201.

brewed on this plan, and another danger is that they usually contain a larger percentage of nitrogenous matters, and this militates against stability. Lager beers are brewed on a system known as the 'decoction' method, the essence of which is that the mashes are made at a comparatively low temperature, and then successive portions of the mash are removed from the mash-tun and raised to boiling, then run back to the original mash till its temperature has been brought up many degrees above the initial heat. The rise of temperature from the initial heat to the final mash-heat is from 30° to 40° F.

Setting aside the extraction by the hot mashing liquor of the substances which exist in a soluble state in the malt—some 20 per cent. of its weight—the chief action that goes on is the transformation of the insoluble starch of the malt by the diastase. This matter has been pretty fully dealt with from the theoretical point of view in Chapter VI. Mashing may be regarded as an operation in which starch is being gelatinized and simultaneously acted on by diastase, which is subject to the modifying influence of the mash temperature. The starch which is earliest gelatinized is transformed by diastase which has had a comparatively short exposure to the prevailing temperature (say 150° F.), whilst the starch, which is the latest to be gelatinized, is acted on by diastase which has had a prolonged exposure to the temperature of the mash (say 150° F.). The diastase, unmodified or less modified by heat, being more active, gives a larger proportion of maltose and less dextrin, the transformation of the starch being carried to a further point; while the diastase at the close of the mashing operation cannot carry the transformation so far, and consequently gives less maltose and more dextrin. The production of malto-dextrin is favoured by the modification or restriction of the action of diastase by heat, but is perhaps more a question of the time during which any given quantity of the gelatinized malt-starch has been operated upon by active diastase: in other words, as to how far the conversion of the starch has proceeded. The average mash temperature (say 150° F.) is one at which a certain amount of combined maltose and dextrin would be found in almost any starch conversion; but the amount varies very much in worts, being governed by various factors, such as the original diastatic power of the malt, the particular grading to which the malt is ground, whether underletting is practised or not, &c.

At first sight the proportions of maltose and dextrin (setting aside malto-dextrin) obtained in ordinary mashes at about 150° F. should

correspond fairly well with C. O'Sullivan's B equation (see page 102), in which the proportions are—

Maltose	67·8
Dextrin	32·2
	100·0

But the large excess of diastase in the mash-tun and the duration of the mashing process determine a transformation of the starch, which corresponds more nearly with

Maltose	75·0 per cent.
Dextrin	25·0 ,,
	100·0

[It must be borne in mind that it is the *starch* of the malt which is under consideration, quite irrespective of the sugars, &c. which are present in the malt before mashing: these are dissolved out in the mashing process almost without change in their nature and quantity.]

By the time that the worts are run off and the sparge started, the transformation of the starch has proceeded pretty well as far as it is likely to; the residual diastase is capable of converting a fresh quantity of gelatinized starch to the same point, but not to a further point of degradation. The net effect of the sparge liquor lies more in the direction of washing out matters already dissolved, but it can, if applied at high temperatures, say 170° F. and upwards, somewhat modify the latter end of the starch transformation in the direction of increasing the amount of dextrin, free and combined. A more important effect of high sparging heats is to dissolve out a portion of the less soluble albuminoids and amides, and no little trouble may result from this, as it tends to give cloudy beers.

The following figures—which are the average of a good many malt samples—may be taken as showing the relationship of the soluble to the insoluble constituents in the wort obtained when average malt is mashed to give an initial heat in the mash-tun of 150° F., and under conditions which would give a tap-heat of not less than 150° F. after 'standing on' two hours:—

Ready-formed sugars, i. e. sugars existing in the malt	12·9
Maltose (free and combined) from the transformation of the starch of the malt	39·5
Dextrin (free and combined) from starch transformed	10·4
Soluble inactive matter largely albuminoids . .	7·6
Moisture in the malt	3·6
Grains	26·0
	100·0

If the solids of such a wort be taken as 100, the foregoing results calculate out as follows :—

	per cent.	
Ready-formed sugars	18·3	= 74·8, total sugars in the wort.
Maltose (free and combined)	56·5	
Dextrin (free and combined)	14·6	
Soluble inactive matter	10·6	
	100·0	

The relation of sugar to non-sugar is approximately 75 : 25 or 3 : 1. The specific rotary power of a wort of this composition—taking C. O'Sullivan's average values for the ready-formed sugars—is 121·3 AD = 133·4 AJ. If the wort, as given above, be fully fermented with active brewery yeast, and the ordinary analytical methods be applied, the beer resulting from the fermentation is found to display the following average composition :—

	per cent.	
Sugars fermented	58·70	72·65, total sugars.
Ready-formed sugars and maltose		
Free maltose unfermented	2·85	
Combined maltose (as malto-dextrin or amylöins)	11·10	
Combined dextrin	4·00	14·1, total dextrin.
Free dextrin	10·10	
Inactive matter	13·25	
	100·00	

We may summarize the effect of variation in the initial heat of the mash as follows, taking 150° F. as a normal or average heat :—

Initial heats below 150° F.—More maltose; less dextrin; less malto-dextrin; more soluble inactive matter; *a more fermentable wort.*

Initial heats above 150° F.—Less maltose; more dextrin; more malto-dextrin; less soluble inactive matter; *a less fermentable wort.*

These summarized conclusions are very plainly borne out by the following results obtained by actual experiments made by the writer in conjunction with Mr. F. E. P. Forster[1] :—

[1] *Proceedings of International Brewers' Congress*, Chicago, Ill., 1893, p. 53.

ANALYSIS OF MALT WORTS FROM EXPERIMENTAL MASHES, BEFORE AND AFTER FERMENTATION.

Expressed as percentages on the malt.

	At 148° F.	At 152° F.	At 156° F.
Extract . . .	75·3	74·8	74·6
Ready-formed sugars .	17·6	17·6	17·6
Maltose . . .	47·2	42·3	38·1
Dextrin . . .	2·0	7·4	13·1
Soluble inactive matter	8·5	7·5	5·8
Grains and moisture .	24·7	25·2	25·4
	100·0	100·0	100·0

Expressed as percentages of the dry solids of the worts.

Attenuation.	1029·2 to 1004·3	1029·5 to 1005·7	1029·4 to 1008·0
Sugars fermented .	66·6	62·5	54·9
Free maltose left unfermented . .	7·0[1]	1·8	3·9
Combined maltose .	7·0	11·5	10·7
Combined dextrin .	4·0	1·9	6·1
Free dextrin . .	1·0	8·1	12·1
Inactive matter . .	14·4	14·2	12·3
	100·0	100·0	100·0

The effect of underletting and decoction methods has been already dealt with, but, briefly recapitulating, we may say that these methods in the early stage favour a high maltose percentage; in the later stages a high dextrin percentage, and the result is to produce a considerable percentage of malto-dextrin of a kind which is slowly fermentable by Saccharomyces Cerevisiae. The presence of these substances causes the beers to drink full on the palate, an effect which is enhanced by the presence of albuminoid matter—probably of the nature of globulin—extracted at the higher temperatures employed.

[1] The fermentation in this case had evidently not gone so far as it could have done. There are some slight discrepancies in these figures if critically compared, but they are not such as to impair the main conclusions that are deducible.

The apparent conjunction of maltose and dextrin in the amylöins, malto-dextrins, or 'reducing dextrins' is one of the most difficult matters to deal with in the chemistry of starch transformation, and it is a complicating factor when one attempts to follow the changes in the mash-tun. Whatever the explanation may ultimately prove to be, we have in practice to do with unfermentable or difficultly fermentable residues containing maltose and dextrin in such a state that

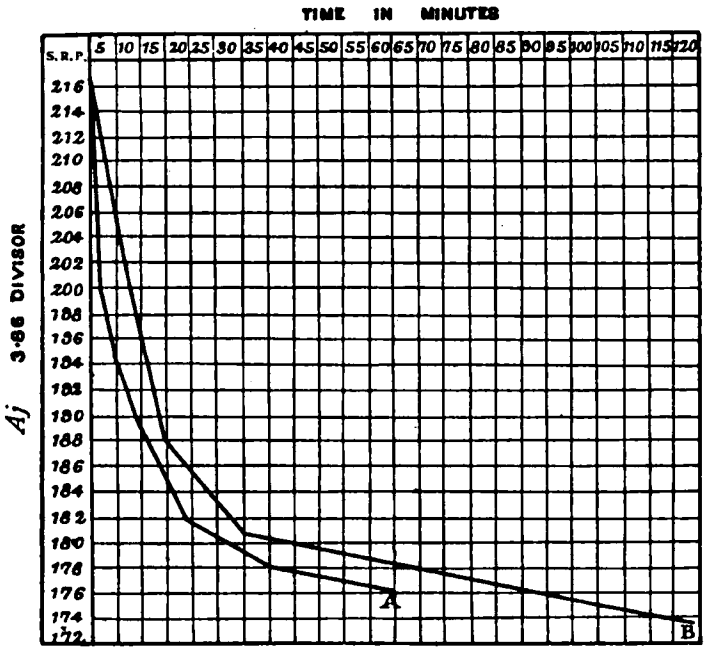


FIG. 22.

A. Transformation with malt extract heated to 66° C. = 151° F.

B. Transformation of starch in brewery mash at 151° F. (2 barrels per quarter; stand on 2 hours).

some fresh cold-water malt extract (i.e. malt diastase) will transform or degrade the substance to maltose only, which is then readily fermentable. The proportion of maltose to dextrin is by no means constant in these residues; it may vary greatly on either side of the ratio, 1 M to 1 D. If the maltose constituent predominates, the malto-dextrin is somewhat more susceptible of fermentation by S. Cerevisiae; if the dextrin portion predominates it is less susceptible of fermenta-

tion. Whatever the kind of malto-dextrin present in a fermented malt-wort or beer, it is much more readily fermented by fortuitous or wild yeast (e. g. S. Pastorianus or S. Coagulatus) than by S. Cerevisiae.

The brewer is placed in the position of requiring to adjust his mashing temperatures so as to secure fairly regular and favourable proportions of the carbohydrate substances alluded to, which proceed from the starch of the malt. The main action in the mash-tun is probably determined in the first hour, and the more finely ground the malt the more quickly does the action take place. The diagram (Fig. 22), arranged from figures given by Mr. Heron in a paper on 'The Polarimeter and its Uses[1],' shows the rate of the reaction very clearly; but the polarimeter values appended appear to be those relating to the starch of the malt only. If the whole of the constituents were under consideration the point of stoppage of the reaction would be somewhere around 120 AD., for the specific rotary power is very much lower than the figure that all starch would give, by reason of the presence of the ready-formed sugars of the malt (see specific rotary power of the carbohydrates, p. 108).

The figure shows how closely the transformation obtained in the laboratory corresponds to the starch conversion in actual practice. Had the laboratory transformation been carried on for two hours it would have stopped at about the same point as the other. The angle corresponds to about 75 per cent. of maltose and 25 per cent. of dextrin.

A very important factor in determining the character of the wort obtained—and one entirely apart from the temperature of the mashing liquor and the resulting initial heat of the mash—is the temperature at which the malt has been dried on the kiln. The following figures may be contrasted: they represent the worts obtained by mashing for two hours at 150° F., the same sample of malt dealt with at very different temperatures on the kiln:—

	A. Green malt 2 days on kiln.	B. Pale malt cured at 190° F.	C. Mild malt cured at 220° F.
Ready-formed sugars	13·5	13·7	13·7
Maltose	59·0	50·0	41·0
Dextrin	13·0	21·5	33·0
Soluble inactive matter	14·5	14·8	12·3
	100·0	100·0	100·0

These figures show most clearly the restrictive action that the

[1] *Journ. Soc. Chem. Industry*, April 30, 1888.

higher temperature exercises on the diastase, and the consequent diminution in the maltose and increase in the dextrin. The total sugars fall from 72·5 per cent. in A to 54·7 per cent. in C, whilst the dextrin rises to a nearly corresponding extent. The wort A would clearly be much more fermentable than B, and B more so than C.

The brewer, then, in aiming at a certain composition of the wort has to take the following chief factors into consideration:—

1. The temperature at which the malt has been dried and cured.
2. The diastatic power of the malt.
3. The initial heat to be worked for in the mash-tun.

And as somewhat subordinate considerations:—

4. The character of the brewing-water.
5. The time of standing on in the mash-tun.
6. The temperature of the sparge liquor.

We have sketched the chief reactions which proceed in the mash-tun. The action of the diastase is the paramount one, but there are also influences resulting from the character of the brewing-water which have been fairly adequately dealt with, we believe, earlier in this chapter. In addition to the reactions which are more or less traceable, there are doubtless certain changes going on in the albuminoid substances parallel, in all probability, to the action of pepsin on albumen, giving rise to nitrogenous nutrients for the yeast employed to ferment the worts.

In the grains is left a variety of insoluble substances, organic (nitrogenous and carbohydrate) and inorganic. Wet grains carry about 60 per cent. of water; when dried, as they not unfrequently are now for cattle-feeding purposes, they show a composition allied to the following figures:—

	per cent.
Starch, hemi-cellulose, residual sugar, &c.	33·0
Woody fibre	27·0
Albuminoids or proteids	16·0
Fat	7·0
Mineral matter (ash)	5·0
Water	12·0
	100·0

The Boiling of the Wort with Hops.

The chief objects to be attained in this part of the process are:—

1. To obtain the necessary concentration and the preservative effect of boiling.

2. To obtain an enhancement of flavour by the cooking which the wort undergoes, due to a certain amount of caramclization resulting from this cooking.

3. To extract the bitter and other qualities from the hops which are added to the wort.

4. To bring about certain chemical changes in the components of the wort which are recognized as necessary for sound and vigorous fermentation, and are thus conducive to the stability of the beers.

First, as regards the preservative effect:—Sweet wort is a liquid of but little stability, and if cooled and exposed to the air it very soon enters into fermentative or even putrefactive change, owing partly to the organisms derived from the surface of the malt, e.g. mould spores and bacteria (cells of Saccharomyces are present on the malt, but they would be killed by the prolonged exposure to a temperature of about 150° F.). Besides the organisms gathered from the malt there is also infection by air-borne germs to be reckoned with. The net result is that even if sweet wort enters into a fortuitous alcoholic fermentation, the bacteria, &c., which are also present cause it to be an unclean fermentation. Again, supposing that the hops were infused in the sweet wort as it came from the mash-tun and the mixture allowed to cool, and the hops strained off, the probability of alcoholic fermentation setting in would be greatly increased, for hops carry, amongst other organisms, cells of Saccharomyces; still it could not be a clean fermentation, as many varieties of organisms would be striving for the mastery in the liquid, and the result would be plainly apparent in the flavour. The prolonged boiling in copper kills all the organisms which have got into the wort up to the point of the copper being full, and the hops in; even the spores of moulds and bacteria, resistant though they are, succumb to the long exposure to a high temperature, and so bitter wort as it comes from the hop-back is practically a sterile liquid.

Secondly, with regard to the great improvement in the flavour of the wort due to boiling. This is no doubt largely owing to the nature of the substances extracted from the hop, but there is another very important side to the matter, and that is the gentle burning or roasting of some of the original constituents of the sweet wort. Comparatively little is known about the chemistry of this action, but in any case it would hardly be necessary to dwell upon it. The action is comparable to that which gives malt its pleasant biscuit-flavour, and indeed the flavour of biscuit itself has, doubtless, its origin in similar changes of some of the carbohydrate and, possibly, albuminoid constituents of the flour.

Thirdly, as regards the substances extracted from the hops, the considerations here are of more importance and worthy our fuller attention. The valuable constituents of the hop reside almost entirely in the so-called 'condition' or lupulin, the yellow glands adherent to the inner side of the leaves: these vesicles constitute on the average some 11·8 per cent. by weight of the hop cone. The leaves, strig, and seeds furnish some tannin and a little fatty matter, and have a certain mechanical effect in helping the 'break' of the worts in the copper; but their chief mechanical action is as a filter-bed in the hop-back. We may, therefore direct our attention more particularly to the components of the hop glands; they are:—

The essential oil.
The true bitter of the hop.
The resins.
The tannin.

The essential oil consists of a mixture of hydrocarbons of different boiling points, the aromatic constituents are probably terpenes, and A. C. Chapman, who has done much valuable work on the subject, suggests the name of ***humulene*** for the terpene which possesses the characteristic aroma of the hop. A. C. Chapman says that from 160 lb. of hops he obtained about 5 fluid ozs. by steam distillation. Some of the hydrocarbon constituents of the oil oxidize readily on exposure to air, forming hard resins, and the oil becomes very viscous in consequence. The oil being volatile in steam, nearly 90 per cent. of the whole is driven off during the boiling of the wort; the small remainder is, however, quite sufficient to give some character to the bitter wort, and an aromatic smell proceeds from the wort during active fermentation, showing that the volatile oil is not wholly dissipated. Attempts have been made to collect the hop oil which is driven off from the wort copper, but the flavour of the oil is impaired by the presence of other substances, and it has not been found profitable. A fresh amount of hop aroma is imparted to beers by putting a small quantity of dry hops, from $\frac{1}{2}$ lb. to $\frac{3}{4}$ lb. per barrel, into the sale cask. This is termed 'hopping down,' or 'dry hopping.'

Although much research has been made into the bitter principles of the hop, it is not yet a matter of certainty what the non-resinous bitter is; one bitter substance, cholin, was satisfactorily identified years ago by Griess and Harrow, but the quantity of this substance in the hop is small. The major part of the clean true bitter is probably alkaloidal in its nature, possibly an acid salt of some alkaloidal base.

The most convenient term for it is the one given by Griessmayer, viz. lupuline. It has a clean strong bitter. A good deal of the bitter of the hopped wort is due to the entering into solution of some of the resin of the hops. Two chief resins have been identified, a hard and a soft resin: the hard resin seems to possess a moderate, and not unpleasant, bitter. The bitter of the soft resin is such as to cling about the palate in a very disagreeable way. The bitter resins exercise a very marked preservative effect on the wort, being inimical to the growth of bacteria, but not to the growth of yeast. The great bulk of the resin dissolved in the worts is eliminated at various stages of the process from the copper onwards; were it not so the beer would be undrinkable from the quantity and quality of the bitter it contained.

Hop tannin, or gallotannin, is present in hops to an average extent of about 4 per cent. It has the usual rough taste of such substances. When hops become somewhat aged, much of the tannin is resolved into a reddish-coloured oxidation-product, somewhat soluble in the wort and possessing an astringent taste. The substance can be separated without difficulty from a strong aqueous infusion of hops.

The somewhat rapid deterioration of hops under ordinary conditions of storage, has led to the introduction of a most successful method of storing them which is coming rapidly into more extended use. This is the so-called cold storage, in which the hops are kept in refrigerated chambers and taken out as required.

The chief points in the use of 'copper-hops' is that they should be of reasonably good quality, and not older than yearlings, the different kinds carefully blended to secure the best possible flavour. They should not be overboiled, i.e. over-extracted, a state of things which is not easy to do with hard waters, but which is only too easily done with softer extractive waters. A bitter may result from over-extraction which can never be removed from the beer even by prolonged keeping. It is a good plan to separate the best hops from the less good ones, and give them a less time of boiling. This tends to conserve the aroma of the better class hops.

Fourthly, as regards the traceable chemical changes in the wort:—

1. The caramelization already alluded to—nature of change uncertain.
2. The coagulation and separation in a flocculent form of albumen. With this coagulum is associated, probably in loose chemical combination, tannin, soft resin and mineral matter mainly calcium triphosphate.

3. The oxidation of some of the matter—resins and tannin—derived from the hop, owing to the free exposure to air during the long boiling.
4. Increase of the acidity of the wort owing to acid substances derived from the hops, e. g. malic and citric acids, and partly, perhaps, from the production of some acid phosphates by the interaction of secondary and tertiary phosphates of potassium, calcium, and magnesium with the organic acids.
5. The interaction of the mineral substances of the brewing water with the mineral matters of the malt. With even moderate amounts of gypsum and magnesium sulphate present, there is doubtless an interaction of these with the soluble phosphates of the malt (mainly potassium phosphate) whereby a marked amount of calcium triphosphate is thrown out, some however being retained in solution, whilst a little hydric magnesic phosphate is eliminated, but much more remains in solution. These reactions have been already described on page 192. We may repeat that the formation of potassium sulphate is of much importance for yeast nourishment.
6. The hops contribute some of their mineral matter to the worts. The average ash of hops is about 7·5 per cent., and potassium and phosphoric acid together constitute quite one-half of it, and the lime (CaO) and magnesia (MgO) one-fifth.

The Hop-Back.

There is but little taking place in this vessel of the nature of chemical change. A further amount of oxygen is taken up from the air and temporarily affixed to certain constituents of the wort. The bulk of the insoluble matters, spent hops, seeds, strig, bits of coagulated albumen in conjunction with tannin and resin, and any small pieces of malt, are retained in the filter-bed which the hops themselves provide.

The Cooler and Refrigerator.

The chief scientific considerations in connexion with these operations are (*a*) the question of aerial contamination; (*b*) any further chemical change in the bitter wort. As regards the first point the English method of brewing, and indeed most other methods taken in

the aggregate, connote a certain amount of infection from the germs which the air brings to the wort on the cooler. The wort comes to the cooler sterile, but directly the temperature falls below, say 150°–160°F., bacteria, and particularly bacterial spores, falling in may retain their vitality; and they have, obviously, the better chance of doing this the lower the temperature of the wort. Long exposure on the cooler and over the refrigerator means a greater number of organisms finding their way into the wort. Quick cooling is therefore most desirable, and this is best secured not by large cooler area but by having a powerful refrigerator.

As regards purely chemical changes, there is practically nothing to remark upon but that a further absorption of oxygen takes place, leading to a further oxidation of some of the wort constituents. Experience leads one to conclude that these oxidation changes take place in the most favourable manner when the scalding wort is rapidly cooled in a thin layer by passing over a refrigerator of what is termed the 'vertical' pattern. The exposure to air is much more complete, and the subsequent effect as regards clarification of the beer is very marked. Attempts have been made to filter the air passing to cooler and refrigerator spaces, but whilst theoretically sound in principle, the methods have failed in the direction of permitting a free and liberal circulation of air. Unless there are known causes of aërial contamination in the neighbourhood of the cooler and refrigerator, such as the escape of malt and barley dust, proximity to dusty roads, manured fields, insanitary dwellings, &c., there is not likely to be much trouble if the worts are quickly cooled, and if other necessary factors of success such as thoroughly good materials, clean and sound vessels, correct temperatures, &c., are paid proper attention to. The great point is to start with a strong and healthy type of yeast, and do everything possible to maintain it in this state.

Fermentation.

The most important part of the brewing process. Mistakes made here have far-reaching effects on the character of the beer. From what has been already said in these pages it will be readily discerned that in the fermentation of a liquid such as bitter wort, we have to do with somewhat complex changes which range themselves under the headings physical and chemical; some indeed may not inaptly be termed physico-chemical.

The chief physical phenomena are :—

1. The growth of the yeast, including its fermentative action and its increase.
2. The rise of temperature to a maximum with a subsequent falling away.
3. The so-called attenuation or diminution of specific gravity owing to the replacement of the comparatively heavy sugars by the specifically light alcohol.

The rate of fermentation as regards rise of temperature and rate of attenuation are measures of the activity of the yeast, and the crop of yeast gathered of the growth or increase.

The thermometer and hydrometer or saccharometer [1] are the means of ascertaining the progress of the fermentation.

The chief chemical changes are :—

Disappearance of the greater part of the sugar with corresponding formation of alcohol and carbonic anhydride and some by-products.

Modification and assimilation of albuminoid matter and of mineral matter by the yeast.

Absorption of loosely combined oxygen by the yeast.

Elimination of certain matters rendered insoluble. Albuminoids, resin, phosphates, &c. (This is in addition to the deposition of original suspended insoluble matters.)

Withdrawal of hop resin by the growing yeast which takes it up as a coating. In order to clearly show the progress of fermentations on the practical scale, we will give the following examples :—

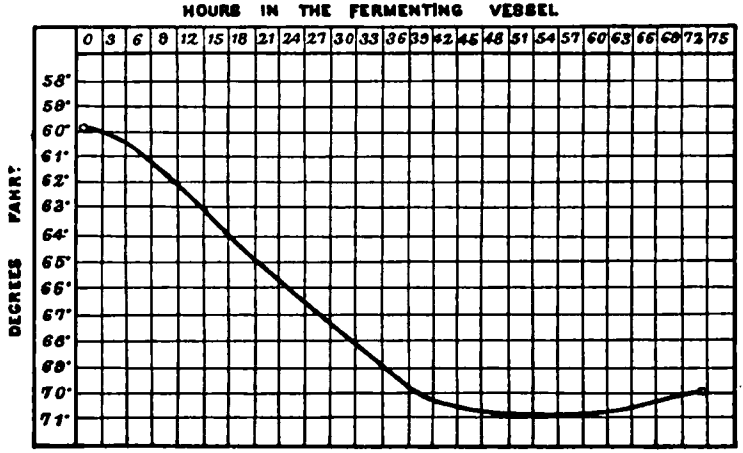
A Typical Skimming Fermentation.

Data.—Wort of 1060 specific gravity gathered into the fermenting vessel at a temperature of 60° F. At a temperature of about 67° F. a slow current of cooling water would be sent throught the attemperators so as to secure a fairly uniform rise of temperature to the maximum point. The first crop of yeast would be ready to skim off at about forty-five

[1] For brewers' purposes the specific gravity of water is regarded as 1000, and the specific gravity of the worts is referred to this (average gravity for worts may be taken as 1055). There is an older and somewhat complicated way of expressing the density of wort which is termed saccharometer or scale weight. The weight is assessed as if a barrel of wort or beer were actually weighed and the excess of weight in pounds avoirdupois over 360 lb. (the weight of thirty-six gallons of water) determined. For instance, a wort of specific gravity

hours, and successive crops at about five hours' intervals: at sixty hours in the fermenting vessel the attenuation is practically at its conclusion, and the temperature begins to be a falling one, which goes on till the beer has come down to about the temperature of the air, or if the air temperature be high the attemperators are kept slowly going till the temperature is reduced to perhaps 55° F., or not exceeding 60° if possible.

Fig. 23 shows the rise of temperature in given time, whilst Fig. 24 shows the rate of attenuation.



SKIMMING FERMENTATION. *Rise of Temperature.*

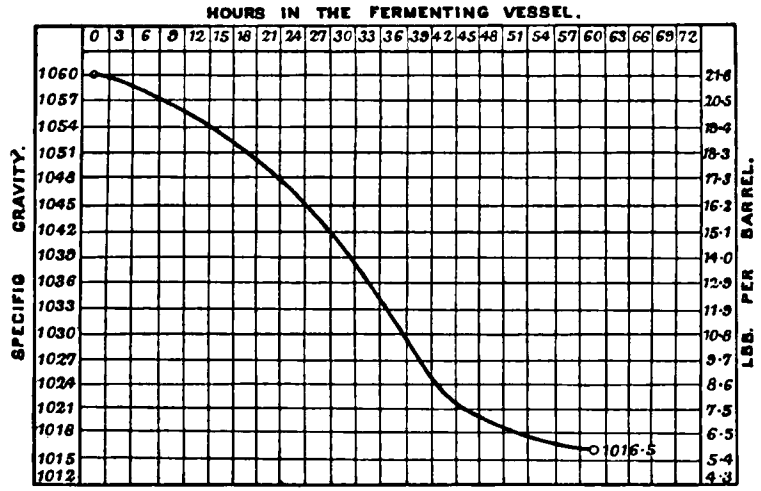
FIG. 23.

It will be seen that the two curves are nearly related till the expiry of about forty-eight hours. Whether the fermenting wort is transferred to a dropping square or the fermentation continued from the point of maximum temperature in the original fermenting vessel, the progress of the temperature records would not differ very materially right away to the racking temperature.

1055 = 19·8 lb. per barrel or saccharometer weight, for a barrel of it actually weighs (360 + 19·8) = 379·8 lb. One degree of specific gravity = 0·36 lb. saccharometer. A beer which has attenuated to say 1014 specific gravity would be spoken of as weighing 5 lb. per barrel (360 + 5 lb. total weight of 1 barrel). Instruments divided according to these two scales of value respectively are in daily use by the brewer.

TYPICAL BURTON FERMENTATION. CLEANSING SYSTEM.

Data.—Two mild ales of specific gravity, 1065 and 1055 respectively. The higher gravity beer was pitched at 58° F. and the lower gravity at 60° F. The course of the fermentation in the fermenting vessel was complete in forty-two hours, by which time the attenuation had reached a point representing the apparent disappearance of about two-fifths of the original solids. At forty-two hours the beers were transferred ('tunned') into cleansing casks or unions. Here the attenuation would proceed till a figure representing nearly one-fourth the original gravity was reached.



SKIMMING FERMENTATION. *Progress of Attenuation.*

FIG. 24.

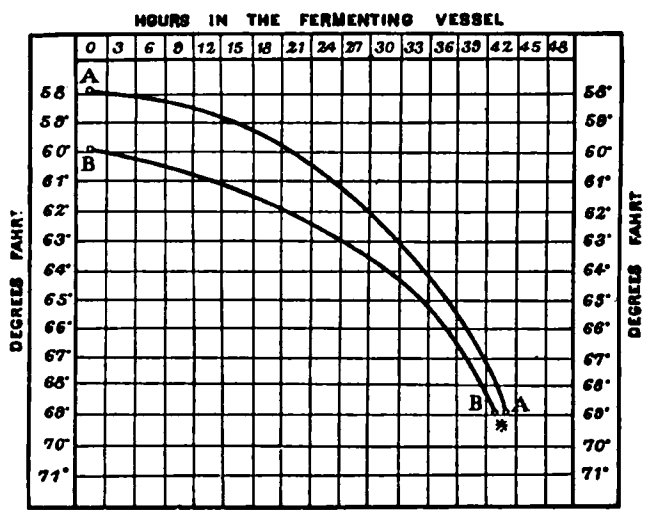
Fig. 25 shows the rise of temperature in a given time.

Fig. 26 shows the rate of attenuation.

In the summer there is no appreciable loss of temperature in the fermenting wort as it passes to the 'union casks' or 'tunners,' and after transference there is a decided tendency to rise in temperature. A rise of more than a degree or two must be checked by careful attemperation, a stream of cold water running steadily through the coils of the attemperators which are fixed into the vessels so as to hold the temperature fairly constant till fermentation begins to slacken. The barm ale and yeast in the troughs should be carefully attemperated

likewise, but not brought to a temperature very markedly below that of the ale in the cleansing casks, so long as they are being fed with barm ale.

The diagrams given display the normal progress of fermentation conducted on two separate systems. The primary fermentation is —on the Burton plan—practically uncontrolled up to the point of the maximum temperature attained in the fermenting vessel, whilst on the skimming system it is attemperated some time before the highest point of temperature is attained. This in itself is sufficient to produce



BURTON FERMENTATION. *Rise of Temperature.*

* *Turned into Cleansing Casks.*

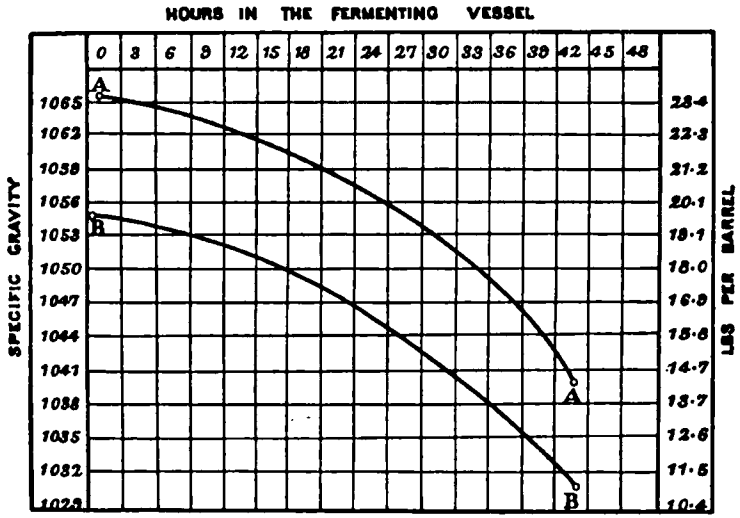
FIG. 25.

rather different types of yeast, let alone other possible differences such as in the brewing-water, the method of yeast removal, &c.

ABNORMAL OR IRREGULAR FERMENTATIONS.

Having paid some attention to fermentations of a normal kind, we may usefully devote some space to the consideration of the reverse aspect of the matter. Nothing is more disturbing to the brewer than an adverse change in the character of his fermentations, and it is

a subject which is particularly interesting from the point of view of possible changes in the nature and vigour of the ferment. The brewer has more control over the materials than he has over the yeast, the latter being, as we know, a living organism with peculiarities of its own. It is also within the power of the brewer to alter his methods of dealing with the materials according to such information as analysis will afford him, but the Saccharomycetes are subject to variations, some of which are not easily traceable to their original cause, and, moreover, the yeast organism proper is subject to the incursion and



BURTON FERMENTATION. *Progress of Attenuation*

Into Cleansing Casks at 42 Hours.

FIG. 26.

detrimental influences of strange or 'wild' yeasts, bacteria, and mould spores. The Saccharomycetes also exhibit very marked variations in habit, with alterations in the aliment, both organic and inorganic, and even small changes in the nutrient medium may, and do, exercise a cumulative influence on the yeast.

Briefly summarizing the chief irregularities of fermentations, we have:—

Stenchy Fermentations. Characterized by evolution of sulphuretted hydrogen and the formation of sulphur alcohols, and possibly other

unpleasant smelling substances besides sulphur compounds. The known causes are—

1. From sulphured hops.
2. From intrusive organisms—wild yeasts and possibly bacteria.
3. From malt carrying an undue amount of sulphur, due to the character of the fuel employed in kilning.

Stenchy fermentations not unfrequently occur in finished beers: this is abnormal 'secondary' fermentation. In most cases of stenchy fermentation, whether primary or secondary, the affected beer usually recovers and becomes an agreeable beverage.

'*Racing*,' *or unusually fast fermentations.* The chief causes are—

1. Setting or pitching worts at too high a temperature.
2. Setting or pitching with too much yeast.
3. A wort containing too large a percentage of saccharine matter, especially of readily fermentable sugars, e.g. dextrose levulose, or cane sugar.
4. Pitching with a very vigorous change of yeast.

The beers resulting from such fermentations generally drink thin, and may remain flat and dull in cask, the fermentable matter being nearly all taken out by the primary fermentation.

Sluggish fermentations. Some of the causes are the converse of those given for racing fermentations. We may, however, state the following:—

1. Weak yeast.
2. Deficiency of fermentable matter and the excess of dextrin probably accompanying it.
3. Want of proper nitrogenous aliment in the worts from poor malts.

Rousing and 'dressing' with some salt and wheat-flour are rather common ways of meeting this particular trouble.

'*Fiery*,' '*boiling*,' *or bladdery fermentations.* Chief causes:—

1. Slack malt.
2. Excess of fermentable matter from overgrown malts of poor quality.
3. Want of viscosity in the worts, absence of globulin, &c.
4. Mashing at too low a temperature; injudicious underletting.
5. Use of sulphites and bisulphites in the mashing liquor, or in the mash.
6. Presence of undue amount of culms in the malt.
7. Hard and steely malts.
8. Excess of malt substitutes, flaked materials, &c.

Some of the causes suggest the remedy or the directions in which to seek it. Dressing with wheat-flour, and in some cases with malt-flour, which must however be used very cautiously, is a palliative; the gluten of the wheat gives some of the fullness which is lacking, and the diastase of the malt-flour accelerates fermentation by liberating some of the combined maltose, and at the same time degrading some of the combined dextrin to maltose.

Yeast Crop or Output.

This is a very important point for the skilled brewer to direct his attention to, for the amount of yeast crop is itself an index to the general well-being of the process, and any departure from the normal crop of any given process points to some important change in the general conditions that govern the process.

The increase of yeast in the slow methods of fermentation is much greater than in the quick ones. The latter are giving a good yield at from three to four times the amount used for pitching (say 1 lb. per barrel or so), whilst in the case of the former five or six times the amount of the pitching yeast is not exceptional. (In the German yeast factories an increase of twelve times the amount of the pitching yeast is secured under the extremely favourable conditions employed.) If the pitching yeast be deducted from the crop, the resulting figure is the new yeast actually formed. It is advisable that every brewer should ascertain what the normal increase is for his average gravity beers: it can be expressed either as the ratio of the *weight* of yeast pitched to the weight gathered, or the ratio of the *volume* of the one to the other: this latter ratio is more easily obtained, but like the former it is subject to some irregularities, according as the pitching yeast carries more or less barm ale. For all-round purposes, a gallon of thick creamy yeast weighs about the same as water, viz. 10 lb. Surplus liquid yeast is often pressed to a semi-dry breakable cake in yeast presses, in which case one gallon of yeast yields a little over 5 lb. of press-dry yeast. The chief factors which influence the amount of yeast crop from a beer fermentation (and for the matter of that from other kinds of fermentation) are:—

1. The specific gravity of the worts. Speaking generally the higher specific gravity worts give more yeast and worts of low specific gravity less yeast than the average.

2. The nature and quantity of the nitrogenous alimentary substances

and the proportion existing between them and the fermentable sugars. There may on the one hand be insufficient sugar to permit the yeast, by exercising its ordinary fermentative function, to assimilate the nitrogenous aliment; or on the other hand the sugar may be much in excess of the nitrogenous aliment, and so the yeast has to put forth a strong fermentative effort without its nitrogenous waste being made good—in other words it does work without being adequately fed, which naturally militates against growth. Good malt is needed to supply good nitrogenous sustenance for the cells.

3. Time of duration of the fermentation. Rapid fermentations tend to reduce the crop markedly below the amount that may be furnished by the same volume of wort under conditions of slower fermentation; this result no doubt has something to do with the larger quantity of oxygen that the wort receives from the air during the more prolonged fermentation. Aeration unquestionably tends to increase yeast crop, acting no doubt by promoting the vegetative habit at the temporary expense of the fermentative habit.

4. Temperature of Fermentation. If within the limit of the optimum temperature of the yeast (see pages 48-9) the action of temperature is referable to time; higher setting temperature means, other things being equal, quicker fermentation, and vice versa.

5. The amount of yeast used for pitching. This only affects the crop materially if the quantity departs far from the amount ordinarily employed, either in the direction of being much less, or much more. If the amount be much less it may be insufficient to promote ordinary primary fermentation; if much more, the fermentation may be most unduly hastened. There is doubtless a general tendency, if proper primary fermentation be promoted, for a given volume of worts to produce the same ultimate weight or volume of crop yeast, whatever the amount of setting yeast may be. This is very plainly shown by the following figures given by Thausing for German beers. These figures have been calculated into English equivalents thus:—

	100 barrels of wort fermented (four different brewings)			
Gallons of	Gallons of yeast obtained as crop			
pitching yeast	A	B	C	D
12	58¾	71¼	62¾	53¾
18	59¾	70¼	70¼	54
24	59¾	56¼	64	55¾

(The average of these figures gives a ratio of pitching yeast to crop 1 : 3·48; two other German authorities give respectively 1 : 3·4 and

1 : 3·7. This constancy of results is due, doubtless, mainly to the uniformity of method and the fact that the specific gravity of the worts varies comparatively little; another reason is the greater uniformity in type of the ferment employed in lager-beer brewing.)

6. The purity and type of the pitching yeast have not a little to do with the amount of crop. The presence of foreign organisms acts prejudicially on the healthy growth and action of the yeast. As regards the type some species of Saccharomyces yield larger crops than others. Some of the wild yeasts, for instance, are very full cropping yeasts, but even the *varieties* of Saccharomyces Cerevisiae vary in this respect among themselves.

Concluding this subject, we may add that the amount of new yeast gathered from a beer fermentation, may be regarded as the direct indication of how much matter has been removed from the worts by the action of fermentation. It should be remembered that the matter which yeast removes would be equally suitable for the nourishment of bacteria if these enemies of the yeast had an equal chance in the wort, and the same remark applies to the mineral aliment, especially the phosphates. Where soft waters are employed it is most probable that some of the nitrogenous matter is in a state better suited to nourish bacteria than yeast, and it is certain that the phosphates are present in far larger quantity than where hard gypseous waters are used as brewing liquor. It seems a direct consequence that soft-water worts should require being dealt with by methods which will ensure a very full crop of yeast being gathered, and thus remove the excess of matters which may, if not withdrawn, go to nourish intrusive organisms in the finished beer.

Cooling Down and Racking.

Considerations connected with the fall of temperature from the period when very little yeast is being thrown up by the beer in unions, tunners, or skimming squares, belong more to the mechanical minutiae of the process, and there is but little of scientific interest associated with them. The final separation of yeast is taking place by the deposition of cells mixed with grounds, and some of the most recently formed yeast still comes to the surface and is visible as a thin creamy skin on the beer in the skimming square or racking vessel. Within some fifteen or twenty hours of racking there should be no more yeast thrown up, and the beer looks still and dark

under the yeasty film if this last be pushed aside. It is preferable that a film of this nature should exist, otherwise the beer may be unduly flattened and lose aroma by escape of the more volatile ethers.

Too much care cannot be exercised in maintaining the cask plant in a sound condition. Casks even in a state of incipient mouldiness are to be dreaded, for they will certainly taint the beer. A really mouldy cask is insusceptible of being restored to sweetness, for, as Lawrence Briant has shown, the mould growth penetrates to a very appreciable depth in the wood, and therefore cannot be reached by the agent which is intended to destroy the growth. The washing out and scalding of casks requires the exercise of much attention. Ales of any standard of excellence usually have some dry hops of good quality and aroma added in the cask ; the pleasant flavours of the hops are absorbed under the conditions obtaining, whilst the coarser flavouring matters remain undissolved. It is now known that the hops contain a substance of the nature of diastase which is able to degrade the malto-dextrin existing in the beer, thus liberating free maltose which is fermentable by the cells of Saccharomyces Cerevisiae which have come along with the racked beer. The dry hops also carry into the beer cells of different kinds of yeast which not unfrequently are the direct causes of secondary fermentation. Beers to which dry hops are not added are usually such as are destined for speedy consumption, and they are brewed so as to contain enough unfermented sugar in the free state to give rise to secondary fermentation almost as soon as they are racked.

Secondary fermentation of a more or less pronounced kind is absolutely necessary for the well-being of beer in cask. Slow and steady conditioning or development of gas is best. When this takes place at the lower temperatures of storage, say about 55° F., a much finer flavour is developed than where the conditioning is more rapid at a more elevated temperature. This applies equally to the storing of ales which have been bottled.

As regards the ferments taking part in secondary fermentation, it is more the exception than the rule for Saccharomyces Cerevisiae to be the predominating species, the reason for this is that it has practically exhausted the wort of the substances with which it can readily deal. Where beers are brewed for long storage there is time for the cells of S. Cerevisiae to become, as it were, acclimatized, and to slowly degrade and ferment the malto-dextrin part of the solids present.

Species of Saccharomyces that figure in secondary fermentation of beer are—

1. S. Pastorianus (two or three varieties).
2. S. Coagulatus (two varieties).
3. S. Ellipsoideus (one variety, possibly two).
4. S. Exiguus (rather rarely).

All these have been fairly closely described in Chapter III.

As regards the risk attending the presence of these wild yeasts, the point of importance is whether their cells predominate in a beer, or whether they are a subordinate growth to S. Cerevisiae, and whether the wild yeast is of one or several species. If the wild yeast is the predominating growth the beer is in a state of 'fret' or abnormal secondary fermentation which may vary from a passing indisposition to a state which prefaces very rapid deterioration; the growth of acid-forming bacteria supervening on the 'fret.'

All errors in the process may induce 'fret'; some of the more marked causes are—

1. Too high percentage of malt made from English barleys alone.
2. Wrong mashing temperatures leading to wrong composition of the worts.
3. Want of vigour in the yeast as regards both fermentative and vegetative power.
4. Undue checking of fermentation by attemperating (this is also a prolific cause of the clinging bitter taste known as yeast-bite).
5. Using a method of brewing unsuited to the water, or vice versa.

PERCENTAGE OF ALCOHOL IN DIFFERENT KINDS OF BEER.

Description.	Original spec. grav.	Alcohol per cent. by weight.
Mild ale	1042	3·9
„	1050	4·8
Pale ale (light)	1055·7	4·9
„ (full)	1062·4	5·4
Mild ale	1073	6·0
Strong ale	1098	9·1
Very strong and old ale	1116	11·14
„ „	1130	12·3
Porter (light)	1052	4·2
Stout (medium)	1075	5·7

CHAPTER XI

Fermentation from the Distiller's Point of View. An Outline of the Production of Alcohol, and Pressed or Baker's Yeast

We have now come to the third great industry associated with alcoholic fermentation, an industry to which a short and comprehensive title can hardly be given, for included in it is not only the process by which any fermented liquid whatsoever may be made to yield up its alcohol; but also the purposeful production of fermentable worts for distillation, either to give rise to potable spirit, or ethylic alcohol; or, in addition to these, the production of baker's yeast. It will be recognized at once that the term 'distilling or distillation' takes no account of the processes prior to the operation it describes, and it is in these earlier processes that by far the larger scope for scientific control is afforded. The manufacture carried on in a distillery is practically brewing (including malting) in its first stage, and distilling in its second; and from the point of view of the science of fermentation the final operation of obtaining the alcohol is of comparatively little interest, as it is a more or less mechanical one, and may be regarded, moreover, as a kind of artificial graft on to processes which are to a certain extent natural in their origin and character. We have just implied that there is much that is common to the two industries—brewing and distilling—but there is one essential difference in the preparation of the respective worts, namely, that the distiller tries to secure as much saccharine matter in his worts as he possibly can, whatever kind of material he may be employing as the source of the sugar—for sugar to him means alcohol: the brewer, on the other hand, has to produce a beverage possessing amongst other attributes a certain amount of palate, fullness, and residual sweetness, and these requirements are incompatible with the production of a highly saccharine wort from which the sugar may be completely fermented out by yeast. Instituting a comparison between a distiller's fermentation and the fermentation of the juice of the grape, we have

this essential difference that in the case of 'must' the fermentation is brought to a termination, quite as much by the fact that the percentage of alcohol has reached a point at which it exercises a markedly restrictive action on the further growth of the yeast organism, as by the exhaustion of the supply of fermentable sugar. [The residual sugar in wines is very variable and constitutes the difference between sweet and dry wines.] Fermentation is the one phase common to all the alcohol industries, and is in each case the most important part of the process, not only because the alcohol, the chief product of the action, is perhaps the main requisite in wine and beer—it certainly is in spirituous drinks—but also owing to the characteristic flavours, practically impossible of imitation, which are simultaneously originated. Another reason why the fermentative stage of these processes is so important, is that a serious divergence here from the normal and recognized lines is sure to be attended by results which are irremediable.

We will now try to classify the different phases in which the manufacture of spirit displays itself in this and other countries. We will pass over the production of alcohol from miscellaneous sources such as rum from molasses, sugar-cane juice, &c., brandy from cider, spirit from fermented fruits such as cherries, &c., crude alcoholic drinks—arrack, vodki—from roughly fermented cereals, &c. There is nothing in the manufacture of any of these worthy of the name of a scientific industry. The manufacture of fine brandy or cognac from wine requires some care and skill, and the product is chemically interesting on account of the variety of higher alcohols, ethers, &c., which it contains, and to which we shall have occasion to make some allusion a little further on.

The spirit industries on anything like a scientifically regulated basis are—

1. The production of potable spirit from cereal sources, which we may subdivide as follows:—

   (*a*) Produced from malt alone. All malt whisky—chiefly manufactured in Scotland.

   (*b*) From malt and raw grain. The majority of Irish whiskies.

2. The production of 'silent spirit' (rectified alcohol) or patent still spirit from cereal or starch-yielding sources. Such spirit may be used for various industrial purposes, and also as a basis for potable spirit such as gin, and for blending with whisky. The manufacture may be thus divided:—

(*a*) Spirit without the production of pressed or baker's yeast.

(*b*) With and including the production of baker's yeast.

Silent spirit or patent still spirit differs in one marked essential from brandy, rum, whisky, &c., in that its flavour is wholly and solely that of ethylic alcohol, and it is required that it should be so. As Mr. Allen puts it, the producer of 'silent' spirit labours to obtain that which does not betray its origin by any characteristic smell or flavour.

Taking a general view of the components of a distiller's wort we have the following:—

1. Sugars derived from starch and other sources, which are fermentable or become so under the conditions of the process.

2. Degradable dextrins which are converted into maltose during fermentation; the maltose so produced is in turn fermented.

3. Substances which directly nourish the yeast organism.

(*a*) Nitrogenous substances, e.g. amides and peptones; also soluble proteids which are chemically changed during the process, and become available as nutrients.

(*b*) Inorganic or mineral nourishments, viz. salts of potassium, of calcium and magnesium, chiefly in the form of phosphates and sulphates.

Taking the carbohydrates separately, which are chiefly concerned, and which are either directly or indirectly fermentable, we have the following:—

Maltose and various dextrins (including malto-dextrin), derived from starch by enzymic action.

Cane sugar.

Dextrose (glucose).

Levulose (fructose).

Cellulose (or hemi-cellulose), (susceptible of being hydrolized by acids into fermentable sugar).

Speaking of distillery operations generally, the chief active natural agents in converting soluble and insoluble unfermentable matters into a fermentable or partially fermentable condition are diastase, cytase, and invertase, also the enzymes proceeding from bacteria. The chief artificial agents are heat, applied with and without pressure, and the influence of mineral acids.

Outlines of the manufacture of all-malt (generally termed 'Scotch') whisky.—There appear to be two distinct classes, known as 'Highland' and 'Lowland'; the chief difference between the two arises from

variations in the method of drying the malt, and in the extent to which the re-distillation or rectification of the spirit is carried.

As regards the production of the malt: a medium or somewhat inferior class of barley is used; it is grown rather rapidly on the floors at temperatures of about 65° F. to 68° F., and when the acrospire is about three-fourths up, it is loaded on the kiln and rather lightly dried, so as to secure a maximum of diastatic activity. Peat is very largely used as a source of heat, and communicates a very characteristic flavour to the malt. Variations in the quantity of peat employed lead to considerable differences in the flavour of the spirit ultimately produced. The drying occupies about two days, and the finishing temperature is comparatively low, viz. under 130° F. As a consequence, the malt comes off the kiln carrying perhaps 10 or 12 per cent. of moisture, but with the diastase comparatively unimpaired. Two examples may be furnished to illustrate this point—

(*a*) Malt dried at 122° F. . . .	88·2	Diastatic activity by Lintner's standard.
(*b*) Another sample dried at 123·5° F. .	85·2	

A malt suitable for the distiller's purposes being thus obtained, it is ground and mashed very much as it would be in the brewery, excepting that the mashing temperatures are kept perceptibly below what they would be in ordinary brewing operations. The heat of the mashing liquor would be about 160° F., which would give a mash-heat of 145° to 147° F. with two barrels of 'liquor' per quarter of malt. 145° F. is a good temperature to work for. The temperature should not exceed 147° F. in the main mash, for if it does, a transformation of the starch may ensue, which will give a not inconsiderable percentage of non-degradable or stable dextrin. If the temperature of the mash is perceptibly below 145° F., there is a risk of acidity setting in from the fostering effect of such a temperature on certain bacterial organisms, especially those which produce lactic acid; besides which, the starch being inadequately gelatinized at the lower temperatures is not susceptible of being properly acted on by the diastase of the malt.

Dr. Squire[1] gives the following figures for the separated starches which are both useful and interesting in this connexion: for present purposes we would draw attention more especially to those which appertain to barley starch.

[1] 'The Processes concerned in the Conversion of Starch into Alcohol,' &c. W. S. Squire, Ph.D., *Journ. Soc. Chem. Ind.*, 1884, p. 399.

	Swelling up.	Commencement of Gelatinization.	Perfectly Gelatinized.
Rye	113·0	122·0	131·0
Maize	122·0	131·0	144·5
Barley	99·5	135·5	144·5
Potato	115·0	138·0	144·5
Rice	129·0	138·0	143·5
Wheat	122·0	149·0	153·5

(Temperatures in degrees Fahrenheit.)

Theoretically it would be advantageous if the mash could stand at a temperature of about 145° F. for many hours, as the transformation of the starch would have been carried to the point of having furnished rather more than 81 per cent. of maltose, and rather less than 19 per cent. of dextrin [these figures are, according to Brown and Heron, about the normal ones for a starch transformation at 140° F. (and according to Dr. Squire at 145° F., compare also Fig. 21 in preceding chapter)], but there are two great objections to such a course: (1) The time consumed, and (2) what is of more importance, the rapid increase in acidity after a few hours, which would more than nullify the favourable effect of time by the injury caused to the diastase. It may be remarked here very pointedly, that the distiller not only needs a quantity of diastase in the mash-tun, but also a surplus of active diastase in the worts, otherwise there is no possibility of the malt-dextrins being degraded andfermented during the fermentation; they would simply be lost.

The mash should therefore stand only a few hours, and then the sweet wort is drawn off. The sparge 'liquor' should be run on at 160° F., then 165° F., and finally 170° F., but it should not exceed this last-named temperature: the sparging is continued till the grains or 'draff' are exhausted of any soluble matters.

Sometimes a method of mashing is employed, which has been termed by brewers, using a similar mode of extraction, 'limited decoction.' It consists of mashing at quite a low temperature—say 125° F. in the mash-tun—and then raising the temperature by successive 'underlets' or 'overlets' of hot liquor, so that the temperature of the mash is brought up to 150° F. or a little higher. The diastase being brought into solution at a comparatively low temperature retains its activity in a full measure, and when the higher temperature softens and gelatinizes the starch the latter is immediately acted on. Diastase is much better able to resist the influence of heat in presence

of carbohydrate matter in solution, and consequently it will resist the higher temperature of the mash, which obtains towards the close of the mashing operation, far more effectually than if a high temperature were employed at the beginning when there is but little matter dissolved.

The worts as they are obtained from the mash-tun are cooled down by refrigerators to the temperature at which fermentation is inaugurated by the addition of the pitching yeast. The specific gravity of the collected worts is about 1,045. Brewery yeast at the rate of at least 1 lb. per barrel of wort is added. The temperature at which the worts are set with yeast is much more variable in distilleries than in breweries, and is much higher: from 70° F. to 80° F. may be taken as covering the temperatures at which wort may be set where the object is to produce whisky. There is no system common to distilleries: each one works to a large extent on its own plan. Owing to the quantity of yeast employed, and the high pitching heat, fermentation commences almost at once, and becomes exceedingly energetic, the temperature rising in a comparatively short time to over 90° F. (94°-95° F. may be attained). In the progress of the fermentation the free sugars disappear at an early stage, and then the diastase liberates maltose from the malto-dextrins, and this is fermented. The presence of diastase, we may repeat, is a most important feature in the proceedings: it directly stimulates yeast-growth; it renders matters fermentable which would otherwise remain unattacked by the yeast, and greatly accelerates fermentation.

There is every reason to believe that a temperature of about 82° F. is the optimum temperature for alcoholic fermentation (see Chapter IV. pp. 48-9); this is greatly exceeded in most whisky distilleries, and the effects are threefold: (1) The deterioration of the ferment employed in point both of cleanliness and activity; (2) the production of higher alcohols and ethers; and (3) to foster the growth of foreign organisms, e.g. bacteria. So far as the production of alcohol goes it would be better that the temperature of the fermentations did not exceed 80° F., but then certain flavouring substances would not be produced, and even if the flavour of the spirit approximated to what was required, the larger amount of time needed would be considered an objection. There is no doubt that a very energetic fermentation is needed to get the worts to attenuate to zero or 'water' (the specific gravity of water is marked as 0·0 on the saccharometer) before acidity gets the upper hand and affects first the diastase and then the yeast.

Butyric, lactic, and acetic acids produced by specific bacteria are the chief causes of trouble. Very small quantities of butyric acid, even 0·02 per cent., will injuriously affect diastatic action. Lactic acid is much less troublesome in this respect, but 0·1 per cent. has a slight effect, and over 0·4 per cent. will markedly restrict diastase, though not inhibiting the action of the yeast. It is plain that if the diastase be injured before the malto-dextrins are degraded, a serious loss of alcohol must ensue, for the yeast alone cannot ferment them. This tendency to increased acidity causes the latter stages of a fermentation to be a source of much anxiety to the distiller. When the temperature of the fermentation is standing at 90° F. or over, and practically the whole of the sugar has been fermented out, the yeast organism begins to enter into the condition known as auto- or self-fermentation, still maintaining thereby the temperature of the liquid, but parting with a portion of its internal matters—a process which not merely weakens the yeast and kills weak cells, but provides foodstuff for intrusive organisms. This is the stage which the distiller watches very closely, for whilst on the one hand a small amount of residual sugar is being transformed into alcohol and carbonic anhydride, another portion of the sugar is being converted into acid, and the alcohol itself may be lessening by conversion into acetic acid by means of the acetic ferment, which is very active at the temperature obtaining. The fermentation being concluded the wort or 'wash' is ready for distillation. In the Highland distilleries this operation is performed in a copper still with a head and a condensing worm of copper, a very simple apparatus known as a pot still (Fig. 27). A stirrer actuated by machinery keeps the solid particles from settling on the bottom of the still and burning there, and so communicating unpleasant flavouring substances of a volatile character. The still is set so as to be heated by an open fire, just as a fire-heated brewing copper is. A small quantity of soap (1 lb. per 100 gallons of 'wash') is added to prevent frothing over. The capacity of a pot still is from 6 to 12,000 gallons.

The products of the distillation are:—

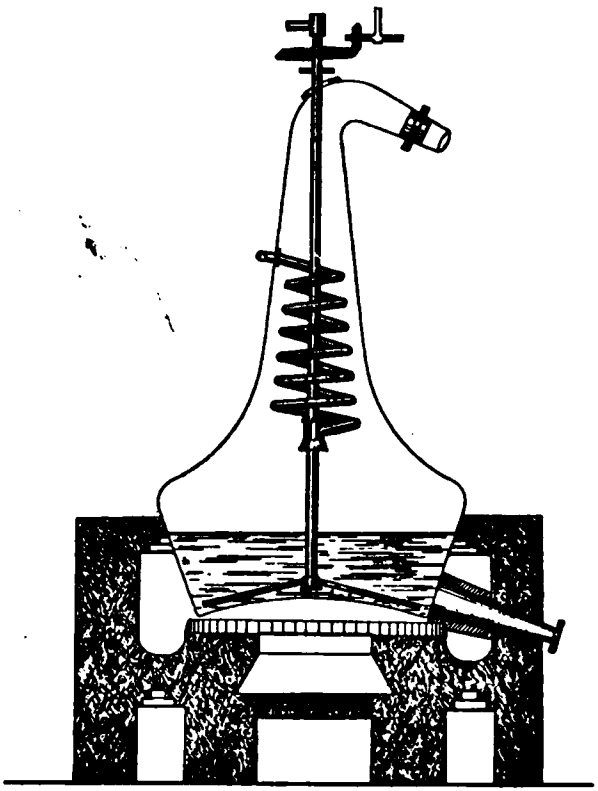
A weak alcoholic liquid called 'low wines' or 'singlings'; and 'spent wash,' 'pot ale,' or 'burnt ale,' which remains in the still and is usually run to waste.

The 'low wines' are redistilled, and yield as follows:—

First distillate, called 'foreshots' (becomes milky when diluted with an equal volume of water); the strength is from 23 to 50 degrees over-

proof[1], which is equivalent to 62·6 to 80·0 per cent. of alcohol by weight. The foreshots are added to the next succeeding 'low wines' and redistilled.

Second distillate, clean spirit or whisky (strength 15 to 50 degrees



IMPROVED POT STILL.

FIG. 27.

[1] Proof spirit or standard spirit is that which, consisting of alcohol and water alone, has a specific gravity of 0·92 (water = 1·0) at 60° F. This mixture contains nearly 50 per cent. of alcohol by weight—actually 49·24. Spirit which is weaker than this strength is called underproof, whilst if it is stronger it is termed overproof. The proportions of alcohol and water by measure required to give specific gravity 0·92 at 60° F. are 100 of alcohol + 81·85 water.

overproof, 57·7 to 80·0 per cent. of alcohol by weight) which is brought in Scotland to a general strength of 11 degrees overproof before it goes into the 'bonded store.'

Third distillate is called 'feints' (becomes milky when mixed with an equal volume of water), the strength of which is about 45 degrees underproof. The feints, like the foreshots, are added to the next succeeding low wines; they contain fusel oil (amylic alcohol) and other of the higher alcohols. The spent lees in the rectifying still are run away: they contain traces of alcohol.

The whisky is then the final distillate.

The disagreeable taste of the newly distilled spirit disappears on storage in cask, chiefly by a selective absorption which the wood of the cask exercises, and also by some little oxidation and etherification of the higher alcohols. An enhancing of the more agreeable flavours is arrived at by storing the whisky in sherry casks, some colour being also imparted thereby.

The method of production of Irish pot still whisky differs chiefly from the manufacture of Scotch in that the malt is not dried over peat, and in the use, by the majority of Irish distillers, of a proportion of raw or unmalted grain which is used in conjunction with malt. Apart from this there is a considerable similarity in the methods, and the same want of uniformity and regularity of procedure. In addition to the ordinary bye-products of fermentation, which contribute the characteristic flavours desired by consumers of whisky, there is very frequently in the case of the Irish product something present—most probably furfurol—which imparts a character of its own. It most probably has its source in the pentosans of the raw grain, for these substances are known to yield furfurol when distilled with dilute acids. It may be regarded as certain that furfurol is present in some Irish whiskies, and when this is the case it is very likely that distinct physiological effects result to the consumer. Speaking of whisky generally, whatever may be the cause of certain physiological effects resulting from the consumption of new or unmatured spirit, it is a matter of ordinary experience that proper maturing does away with them, and there can be no question that this is due either to change of objectionable substances into non-objectionable, or removal by absorption into the wood of the cask. Both influences are doubtless at work, and the results due to the latter are far more distinguishable by analysis than those due to the former. A very interesting and ingenious process, and one, moreover, which throws no little light upon

the matter, is the one invented by Mr. R. C. Scott, of Liverpool. The method consists essentially in cooling the spirit considerably below the freezing-point of water by means of a suitable refrigerating apparatus, and then throwing it into spray in a closed chamber by means of a jet of air which has been filtered through cotton wool, washed with water, and then cooled below the freezing-point, which dries the air by freezing out the moisture. The spraying continues for from four to six hours, the air passing away through a series of pipes cooled so that any alcohol spray mechanically carried over, or any vapour of alcohol are saved. The waste air has a strong disagreeable odour, thus proving that objectionable matters are being removed. No appreciable loss of bulk occurs, and no alcohol is lost. The air doubtless removes some very volatile constituents of the spirit which have an unpleasant taste and smell; some of the higher alcohols must, in addition, be either carried off in some way or oxidized, for it has been shown by analysis that by this process they can be reduced to one-third the amount originally present. Mr. Allen[1] says in effect that he does not regard Scott's system as replacing the ordinary method of maturing in cask, but it will very materially reduce the time required for subsequent ageing, by removing from the spirit at the outset the greater part of the substances which give to raw spirit its nauseous taste and flavour.

Patent Still Spirit.

We now pass on to the processes employed in the production of rectified ethylic alcohol or spirit of wine, and will then outline the Continental manufacture of patent still spirit combined with the output of pressed or baker's yeast, for the combining of the two is in this country rather the exception than the rule. It must be confessed that it is only in a few cases in the kingdom that scientific knowledge and experience have been brought to bear in such a way as to lead to the production of yeast suitable for bakers' purposes, which can compete on equal terms with that which is imported in such large quantities from the Continent. There would appear to be still a fruitful field open in this country for the production of so necessary an article.

Various sources of starch—more especially cereal sources—are made use of by British distillers of silent spirit. The following will serve as an example of a scheme of quantities :—

[1] 'The Chemistry of Whisky,' *Journ. Fed. Insts. Brewing*, 1897, p. 46.

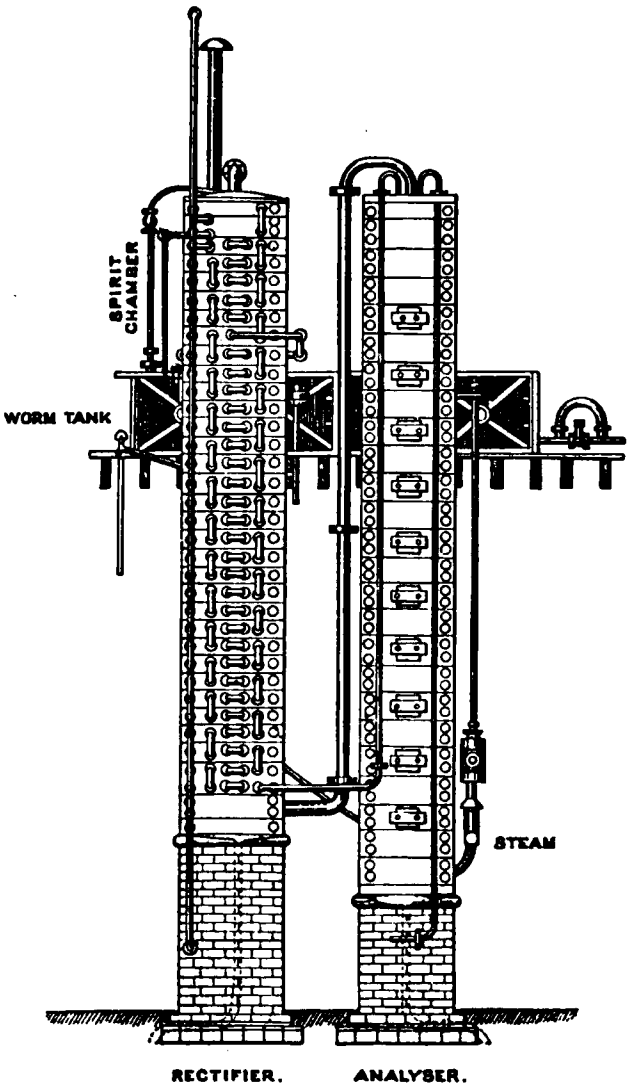
Malted rye .	9·75	parts in the mash-tun.
Ground oats .	7·60	„ „ „
Ground maize	82·65	„ treated separately and added to the mash.
	100·00	

The method of procedure is as follows:—The maize mixed with water and a very small quantity of malt is heated first at a temperature of 150°F. falling to 130°F.: the mixture is then raised by steam heat in a closed vessel and submitted to a pressure of steam of 15 lb. per square inch. It is then cooled to between 150 and 160°F., and used to mash the other part of the grist. The mash heat is about 150°F. falling to 145°F. Stand on a few hours and run off rather thick worts of about 1030 specific gravity; these are cooled and collected in the fermenting vessels and are pitched with about 1 lb. of yeast (pressed brewer's yeast) per barrel of wort at a temperature of 80°F. Vigorous fermentation quickly sets in, and the temperature runs up to 90°F. and keeps at this for some hours. Attenuation goes on very rapidly up to the maximum temperature attained, then falls off, but is still progressing slowly on a falling temperature. The whole fermentation lasts seventy-two hours. The acidity of the fermenting liquid increases rather rapidly during the latter stages of fermentation. The intention, not always realized, is that the worts shall attenuate to 0·0 or 'water.'

In the distillery in which this fairly typical method was pursued, the fermentations were of the kind known as boiling fermentations (they usually are so in worts of this character and low specific gravity), and the yeast was so exhausted by the rapid fermentation in weak worts and without aeration that it was practically valueless, and fresh pitching yeast from external sources was necessary for each run of worts. The spent wash had a specific gravity of about 1,004, and an acidity equal to 0·54 lactic acid (part of the acid was acetic and part butyric).

The fermentation being complete we may now proceed to briefly describe the method by which the spirit is obtained. Coffey's patent still is the apparatus in general use, of which we cannot do better than quote Mr. Nettleton's description[1], and reproduce the illustration which accompanies it. The apparatus consists of two columns, the analyser and the rectifier (Fig. 28). The steam enters the lowest compartment of the analyser, rising up through that column, over it by means of the feints-vapour pipe, and then enters the lowest com-

[1] *Trans. of the Inst. of Brewing*, vii (1893–4), p. 171.



COFFEY'S DISTILLING APPARATUS

(BY PERMISSION OF MESSRS. BLAIR, CAMPBELL AND M'LEAN)

FIG. 28.

partment of the rectifier, in which column it still continues to rise. When the apparatus is heated throughout, the 'wash' is forced through the long zig-zag pipe which traverses every compartment of the rectifier. The pipe quits that vessel at the lowest compartment and discharges the wash into the highest compartment of the analyser. In each column there is a number of superimposed compartments, divided in each case by a strong copper sheet drilled with perforations throughout. Each sheet is also fitted with one, two, or more drop-tubes, supported in place by a strong rim, and fitted one inch above the level of the plate it quits. These drop-tubes discharge into rimmed cups below them. In this way the liquid descends each chamber and overflows on to each plate successively, whilst steam is blowing up through the wash and carries with it the alcohol vapour, so passing together through the perforations. The spent wash discharges from the bottom of the analyser. The hot feints formed during distillation are returned to the top column of the analyser from a vessel connected with the lowest compartment of the rectifier.

The upper chambers of the rectifier constitute the spirit chamber. The spirit quits the lower part of this and runs to the condensing worm. The plates in this portion of the still are not perforated, but have only a circular opening for the condensed spirit to fall down through. The condensation of the alcohol vapour is nearly all effected by the zig-zag wash-pipe, the temperature of the wash being low enough to permit of this. The rectifier is fitted at the top with another pipe which leads to the condensing worm, and with an escape steam-pipe in case of accidents. The strength of the spirit obtained is about 68 degrees overproof, which is =93·8 per cent. of alcohol by weight. The principal feature of patent-still distillation is the practical elimination of all flavouring constituents in whatever quantity they may have existed in the fermented wash. The existence of these bye-products and their nature can be best considered in connexion with other distilled alcoholic liquids, e. g. brandy and whisky. We may clear the ground somewhat by stating that the very large number of alcoholic and ethereal bye-products may be divided into two groups. One group containing substances whose taste and smell are pleasant and fragrant, or at least not objectionable; in the second group may be included the substances which are of usual occurrence in, and which constitute the chief part of so-called fusel oil. The only exception to this that need be made is, that very small quantities of the objectionable bye-products may be present in a potable spirit

without doing much else than helping to constitute a combined characteristic flavour—the pleasanter-flavoured substances dominating the less pleasant ones. By distilling a large volume of cognac brandy twenty-five years old Ordonneau obtained the following substances :—

	Grains weight per gallon.
*Normal propyl alcohol	28·0
* „ butyl alcohol	153·0
*Amyl alcohol	58·7
Hexyl alcohol	0·4
Heptyl alcohol	1·0
Ethyl acetate (acetic ether)	24·5
Ethyl propionate, butyrate, and caproate	2·1
Oenanthic ether	2·8
Aldehyde	2·1
Traces of other substances	—

The total of the substances named = ·39 per cent., a very small quantity. The remainder of the constituents of the brandy would be ethylic alcohol, water, and a little solid flavouring matter.

The substances marked with an asterisk are components of fusel oil, as will be presently seen.

Samples of actual fusel oil from brandy[1] :—

	Clandon and Morin.	Ordonneau.
Propyl alcohol	11·9	11·7
Normal butyl alcohol	49·3	63·8
Isobutyl alcohol	4·5	0·0
Amyl alcohol	34·4	24·5

These results will give the clue as to the unpleasant flavoured components of spirit which include the three chief components of fusel oil derived from other sources (such as malt and grain spirit, and spirit from miscellaneous sources, including potato spirit), viz. propyl alcohol (C_3H_7Ho), butyl alcohol (C_4H_9Ho), and amyl alcohol ($C_5H_{11}Ho$).

We may give the boiling-points of some of the substances mentioned :—

		Ethers, &c.	
Ethylic alcohol	78° C.		
Propylic „	97° C.	Acetic ether	74° C.
Isopropylic alcohol	85° C.	Butylic „	112° C.
Butylic „	115° C.	Aldehyde	70° C.
Amylic „	130° C.		
Isoamylic „	120° C.		

[1] From 'Chemistry of Whisky,' A. H. Allen, *Journ. Fed. Insts. Brewing*, 1897, p. 35.

Patent still spirit is, as we have said, practically free from higher alcohols, and the amount of these substances in commercial whisky does not—according to Allen—average more than about 100 grains to the gallon, or a little more than 0·1 per cent. Small as the quantity is its influence on the flavour is very great indeed.

All the bye-products of distillers' fermentations—the higher alcohols, ethers, and acids—have a peculiar interest from the point of view of the chemical actions that give rise to them. They are partly due to the high temperature at which fermentation is carried out, and the constituents of the worts employed. The compound ethers are more particularly due to intrusive organisms, e. g. bacteria, which producing acetic, butyric, lactic, and other acids, give rise to corresponding ethers. Another factor, which undoubtedly has some influence on the nature and quantity of the bye-products of fermentation is the particular variety or species of yeast employed. The lower members of the family of organic acids have a very restricting action on yeast, viz. formic, butyric, valeric, caproic, especially the second and the last-named: 0·05 per cent. of either of these will injure a fermentation, whilst 0·1 per cent. completely spoils it. The following figures, relating to the action of the alcohols on fermentation, given by Kayser [1], are very interesting in the same connexion. The following quantities are those which will stop fermentation:—

Methylic alcohol	2	per cent.
Ethylic „	15	„
Propylic „	10	„
Butylic „	2·5	„
Amylic „	1·0	„
Caproic „	0·2	„
Caprylic „	0·1	„

The Continental System for the Manufacture of Spirit and Pressed Yeast (*Presshefefabrikation*).

The general outlines of the process are as follows:—

1. The preparation of a preliminary mash or wort for cultivating the mother yeast. This is called the 'Hefegut.' It requires to be from all malt—kilned, air-dried, or green, and to be a rich, strong wort.

2. The Hefegut is artificially soured at a temperature of 122°F.–125°F.—a temperature most favourable to the growth of Bacterium Lactis. The wort may be infected by adding a little sour milk, but

[1] *Les Levures*, p. 159.

this is not absolutely necessary. The souring is allowed to proceed till the lactic acid reaches 0·6 per cent. or over.

3. The temperature of the Vormaisch or Hefegut is raised to a point at which the lactic acid producing organisms are killed; about 155°F.–160°F. suffices to destroy the bulk of them.

4. The Hefegut is cooled down, and set with good strong yeast reserved from a previous fermentation.

5. The yeast in the Hefegut being ripe (which is when the new cells have attained the same size as the mother cells) a quantity is taken off to be held over for setting the next Hefegut.

6. The remainder of the Hefegut, in a state of active fermentation, is used for pitching the chief mash or the worts derived from it: or the remainder of the yeast obtainable by skimming is so used, but this is not so usual.

The Chief Mash.

This term is applied to the preliminary treatment of the raw and unmalted materials, so as to gelatinize the available starch, to loosen up the whole texture of the various substances treated, and prepare them for the action of diastase in the mash-tun. Machinery for such purposes has been brought to a great pitch of perfection in Germany, and at least half a dozen excellent and well-proved kinds of conversion apparatus are in use, in which the raw material would appear to be under very complete control during its treatment by steam heat under conditions of pressure and with the necessary agitation or rousing. Some of the machines seem to be beautifully adapted for their purpose, and would probably, if introduced into this country, be found to be very valuable appliances.

Heat and pressure together secure a perfect liquefaction of the starch, and the bringing into solution of a far higher percentage of albuminoid substance. Pretty nearly every starch-containing plant which is common enough to be reasonably cheap has been tried on the Continent for distillery purposes, not only experimentally, but in most cases on a practical scale. Artichokes, buckwheat, chicory, lupins, locust-beans, vetches, sugar-beet, horse-chestnuts, figs, &c., have all been laid under contribution for alcohol production: whilst the ordinary cereals, with maize, rye, and rice, and the potato, are in regular use. The percentage of moisture in these substances is an important matter as affecting extract: the range is very wide, taking

the cereals at about 12 per cent., and the potato at 75 per cent. It may be gathered that the extract value of any given material, apart from its moisture and any sugar it may hold, is practically a measure of the percentage of starch which it contains.

A few words respecting the preparation of malt for foreign distillery purposes. The *sine qua non* is that the malt should be highly diastatic: it is made from reasonably good barley (or rye). Märcker gives the following equivalents:—

100 lb. of barley give 130-150 lb. of green malt.
" " 90-92 " air-dried malt (*Luftmalz*).
" " 80-84 " kilned malt.

All these forms are used in conjunction with the raw and non-malted materials. Green malt and lightly dried malt are largely employed, owing to their powerful diastatic action. It is found that, notwithstanding the large percentage of moisture, a given weight of green malt has the same diastatic effect as an equal weight of kilned malt. As an illustration of this the following example may be given:—It is possible to obtain the available extract of 100 lb. of potatoes in the form of alcohol by means of 7 lb. of green malt, the outcome of 5 lb. of barley. Of this malt 2½ lb. to 3 lb. are needed for the Hefegut and mother yeast, whilst the remaining 3½ lb. to 4 lb. are employed for the actual saccharification of the potato starch. We have now an idea of the possible constituents of the main mash of which the treated raw material forms part. Saccharification is allowed to proceed for 1½ hours to 2 hours at 145°F. The mash is cooled to a temperature consistent with the specific gravity at which it is intended to work, and—according to the older methods of procedure—the whole mash, or at best very rough worts from it, is got into the fermenting vessels. Here the Hefegut with its content of free acid and active yeast is added, and fermentation is thus initiated. Sometimes the Hefegut is added to the mash while the latter is still hot, but in this case the active yeast has been skimmed off, and is added subsequently. In England the worts are commonly separated from the 'goods,' but this—although practised in connexion with the modern Luftheſe process—is rather the exception on the Continent, and doubtless so far as the production of silent spirit is concerned there is much economy in the practice, as a more vigorous fermentation is secured, and a larger yield of alcohol. There is the mechanical difficulty, however, of separating the yeast from 'lees' and 'grounds'—a troublesome operation involving much sieving, settling, washing, &c.

In the foreign method the addition of the sour Hefegut largely protects the fermentation against bacteria, by making the yeast more vigorous and by introducing organic acids which have a directly inhibitive effect on acid-producing bacteria. This is a most important feature in the process. Mineral acids, especially sulphuric, and lately hydrofluoric acid, have been tried instead of lactic acid souring, but although they are of advantage it seems generally agreed that lactic acid is the best. Lactic acid has a most beneficial effect on the yeast as regards colour, stability, and fermentative vigour: it must not be forgotten however that, whilst so favourable to fermentation in quantities up to say 0·5 per cent., it distinctly impairs diastatic activity if present to the same extent in a mash.

Fermentation.

The worts having been gathered from the mash, either by running off or by filter-pressing the goods, or a thick wort containing most of the suspended matters of the mash having been transferred to the fermenting vessels, the yeast is added. The pitching temperature for weaker worts—say of about 1,045 specific gravity—is about 80° F., whilst for strong worts of 1,080 specific gravity it would be about 60° F. The quantity of yeast is proportioned to the strength, but averages about 2 lb. per 100 lb. of materials employed, and is, as already stated, either derived from the Hefegut or Vormaisch, or is included in and added with it. Where the yeast has not been specially cultivated, pressed yeast is sometimes used for pitching, but only in cases of necessity. It is most important that the pitching yeast should be ripe, clean, and active; and it is better, Märcker says, a little too old than too young. A store yeast obtained by high temperature fermentation should be restarted at a comparatively low temperature, as this permits it to make good its loss of nitrogen: but if the yeast becomes too rich in nitrogen—a state of things not likely to occur in the lower gravity worts—there is the attendant disadvantage of a probable falling off in assimilative and reproductive power. The preservation of the nitrogen percentage at an average figure is what is best to aim at.

The fermentation of distillers' worts of fairly high gravity may be divided into three periods—

1. Pre-fermentation. Assimilative and recuperative period of the yeast.

2. Chief fermentation. Fermentation of the free sugars, and action of the diastase on malto-dextrins, forming maltose.

3. Fermentation of liberated maltose and residual sugar generally.

The worts having been properly pitched with yeast, the modern procedure is to pump a gentle stream of air through the worts by means of a suitable apparatus. The stream is so regulated as to be gentle in the early stages of fermentation, and stronger as the fermentation accelerates. The chief effects of this supply of air are:—

1. To agitate the worts and keep the yeast in suspension.

2. To strengthen the yeast as a ferment.

3. To largely increase the crop. (Durst[1] says that aerated worts give three times as much yeast as non-aerated ones, and his statement is amply supported by Pedersen's and Hansen's results.)

4. To control to some extent the rise of temperature due to fermentation.

5. To exercise an influence on the yeast whereby the high temperature to which the yeast organism is exposed for a lengthened time, is rendered practically non-injurious.

Yeast produced under these conditions is known in Germany as *Lufthefe*. Flocculént or coagulated yeasts are not infrequently produced by this method, but they probably result not so much from modification of the nature of the original setting yeast as the introduction of fresh species of yeast by aërial infection. Some of the German Lager-beer yeasts are naturally flocculent in character.

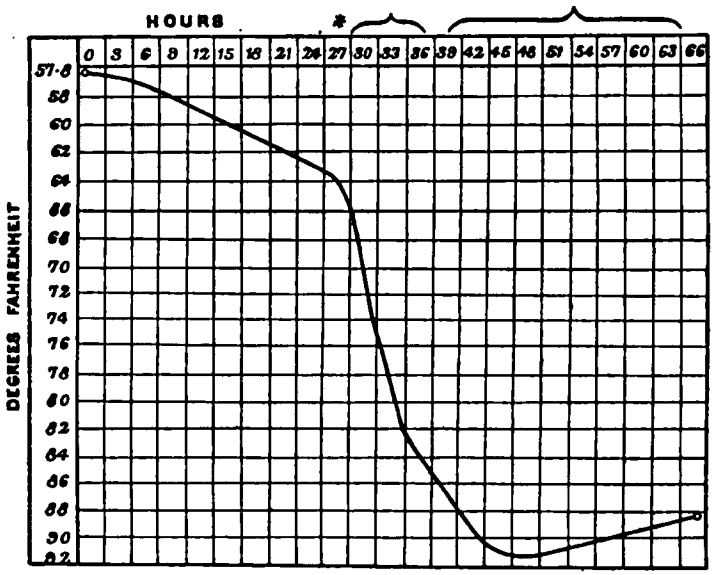
In the most modern distilleries the air which is pumped into the worts is filtered and washed.

The following will serve as the first example of a distiller's fermentation aerated:—

Time (hours).	Temperature.	Spec. grav.	
1	82·0° F.	1048·8	add first half of pitching yeast (*Anstellhefe*).
2	83·7° F.	1050·9	second half of *Anstellhefe*.
3	86·0° F.	1038·3	
4	86·0° F.	1030·2	
5	86·0° F.	1024·0	
6	86·0° F.	1020·0	
7	86·0° F.	1016·0	
8	86·0° F.	1012·0	
9	86·0° F.	1008·0	
10	86·0° F.	1003·2	worts pumped on to settling trays.

[1] *Presshefefabrikation*, Otto Durst.

The next following diagrams from data derived from Märcker's *Spiritusfabrikation* afford good examples of the course of a distiller's fermentation. Fig. 29 shows the rise of temperature during the progress of time by three-hour intervals: the brackets divide off the periods of chief or 'stormy' fermentation and of after-fermentation. Fig. 30 represents the same fermentation from the point of view of attenuation in a given time. It will be noticed that the curves in the two diagrams are practically the inverted counterparts of each other,



* *Commencement of violent fermentation.*

DISTILLER'S FERMENTATION. *Rise of Temperature.*

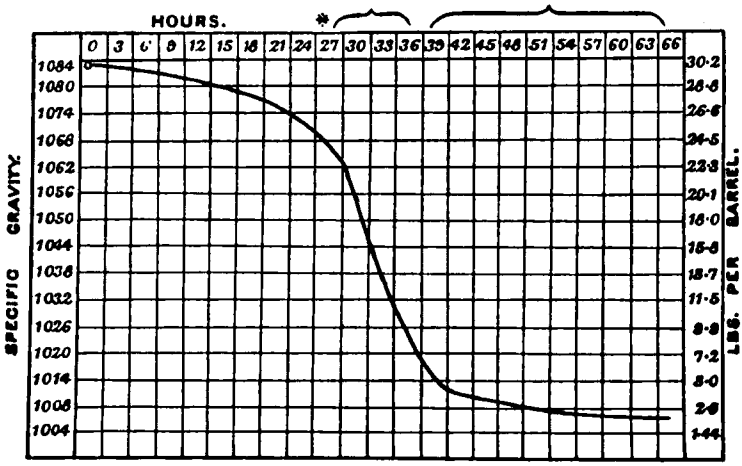
FIG. 29.

showing how closely the attenuation marches with the increase in temperature. The great range covered by both temperature and attenuation records should also be noted.

We will now give an example of a regular fermentation for which Stenglein is the authority (the data for attenuation are not given) (Fig. 31). In this case the wort was a strong one, containing 20·5 per cent. of solids, equivalent to a specific gravity of about 1085. The wort contained 0·5 per cent. acid.

A very interesting comparison may be instituted between these fermentations and the brewery ones given in the preceding chapter.

It is very desirable that distillers' fermentations should not exceed 90° F., and the pitching temperature for strong worts should be adjusted so as to secure this result. Temperature does not much influence the crop of yeast if kept within proper limits, and if the fermentation be complete, but it does affect the rate of fermentation very markedly. The quickest increase in the number of yeast-cells



* *Commencement of violent fermentation.*

DISTILLER'S FERMENTATION. *Progress of Attenuation.*

FIG. 30.

in a given time is, we know, greatest at from 82°–86° F. (Chapter IV. pp. 48, 49.)

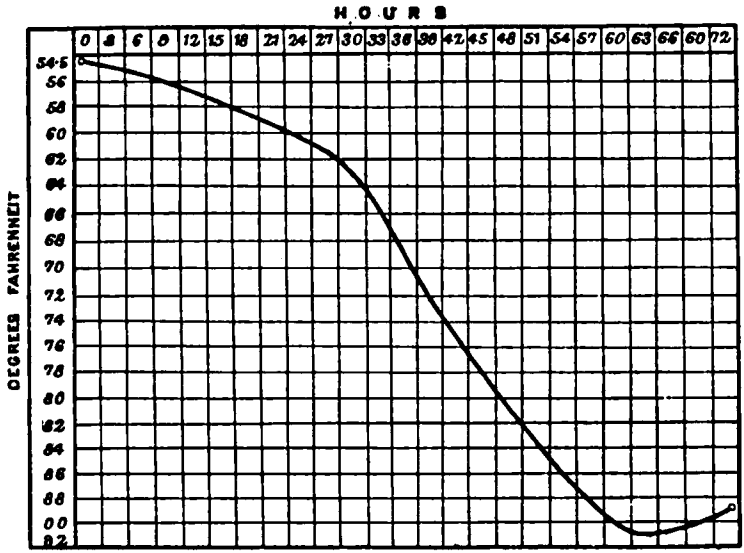
The different kinds of fermentation occurring in Continental distilleries are thus described by Märcker:—

Cover Fermentation is so named when the grains and particles of a thick fermented mash rise to the surface. This is considered a bad sign when potatoes form a large percentage of the materials. The fermentation should be vigorous enough to keep the suspended matter moving about.

Rising and Falling Fermentation behaves as the description implies, accompanied by the evolution of unpleasant smelling gases.

Generally associated with very thick worts containing a low percentage of saccharine matter from materials poor in starch.

Revolving Fermentation. Practically the same, no doubt, as a boiling fermentation. The evolved gas rises from the central portions, and the bubbles run toward the sides, and to some extent under. This in a distiller's wort of low specific gravity is quite a normal fermentation, and is regarded as satisfactory. It would certainly not be so regarded by a brewer.



REGULAR DISTILLER'S FERMENTATION.
(AFTER STENGLEIN)

Wort of 20·5 per cent = 1085 Spec. Grav. Acidity = 0·5 % as Lactic.

FIG. 31.

Foaming Fermentation is productive of great inconvenience, owing to the unmanageable head of foam or froth. The cause is uncertain and debateable. It generally sets in with the chief fermentation, and tends to disappear at the close of it. It comes on at a temperature of about 77° F., irrespective of the pitching temperature, at an attenuation equal to about one-half the original specific gravity. Delbrück maintains that it is not a harmful kind of fermentation, and is in many cases associated with some peculiarity in the composition of the potato used

in the process. It has, however, been observed with wheat and Hungarian maize.

The fermentation is carried to as low a point of attenuation as can possibly be reached without keeping the worts in the fermenting vessels for a period that would entail risk of loss by acidification. During the progress of the fermentation, yeast is skimmed off at proper intervals, and this yeast—subtracting a small amount of store yeast for succeeding operations—becomes eventually the 'pressed or baker's yeast of commerce.' If the yeast is not skimmed off, it is obtained by settlement from the fermented worts. We will, in the briefest manner, outline the subsidiary methods by which pressed yeast is produced.

1. Running the worts on to shallow settling trays, in which the yeast settles and can be separated out: this is done with weaker worts where the yeast is not (and generally cannot be) removed by skimming; otherwise the yeast is skimmed off the surface of the fermenting worts.
2. Washing the yeast with water at about 50° F. Two washings usually suffice, with a volume of water equal, each time, to about one-half the volume of the fermented wort from which the yeast proceeds.
3. Sieving by hand or machine to remove suspended matters other than yeast—wire sieves and silk sieves are generally employed. Fractional settling of the yeast during washing will also free it from some of the foreign matters.
4. Settling the washed and sieved yeast in suitable vessels and separation of the bad-coloured yeast from that which is clean and of the right colour.
5. Pressing the settled yeast in filter-presses with an admixture of starch to secure a drier yeast and so increase the stability.
6. Packing into bags for sale.
7. Storage in cold chambers till removed.

The question as to whether any given yeast will serve the purpose of a baker's yeast depends more upon the natural character of the yeast itself than the way in which it is dealt with in the pressed yeast factory. Some species of yeast possess the power of fermenting dough very strongly, whilst other varieties are almost useless for the purpose. The so-called wild yeasts (i. e. yeasts other than S. Cerevisiae) are usually good panification ferments.

Considerations relating to the Yeast and Alcohol Yield.

A most important consideration for the distiller is the amount of alcohol obtainable from a given quantity of sugar. We have seen that ordinary fermentation entails a loss in bye-products (glycerin, succinic acid, &c.) equal to 5 or 6 per cent. of the sugar fermented. With the sugars which are hydrolized prior to fermentation there is a gain in weight nearly equivalent to this loss. The following figures will show the theoretical yield that the fermentation of the different sugars should give in the distillery: it is almost needless to say that they are not realized, but every effort is made to obtain as high a yield as possible.

1 kilogram of the following substances yields—	Theoretical Alcohol per cent. by Weight.	Theoretical Alcohol per cent. by Volume.	Corrected to 6 per cent. loss.
Dextrose, crystallized	46·46	58·59	55·07
„ anhydrous	51·11	64·45	60·58
Cane sugar	53·80	67·84	63·77
Starch (converted into sugar)	56·78	71·60	67·30

In practice, taking the quantity of spirit obtainable from 100 lb. of starch, the following results may be arrived at:—

	Per cent. of the theoretical yield of alcohol.	Obtd. per cent. of a litre of alcohol per kilo of starch.
Good result	85·1	60·5
Average result	76·7	55·0
Bad result	67·2	48·0

One kilogram of starch yields theoretically 716·12 c.c. of alcohol, or 71·612 per cent. of 1 litre. Calculated to English weights and measures, 1 lb. of starch yields theoretically 0·5678 lb. of alcohol or 11·45 fluid ozs. [The specific gravity of absolute alcohol = 0·793.]

From the theoretical yield of 71·612, 5-6 per cent. has to be deducted for the ordinary bye-products, and a further percentage for loss by formation of 'higher alcohols' and ethers, and loss by evaporation, so it will be easily discerned how a theoretical amount of 71·61 can be diminished in practice to 60·5 per cent., and that be considered good.

The loss by fermentation is greater in clear worts than in thick ones,

chiefly from the compensating effect of the more economical use of the materials where thick worts are fermented, and possibly, too, from the presence of more yeast food leading to less bye-products from exhaustion of the yeast. Losses may be also reduced by attemperating in the fermenting vessels, and so preventing the temperature rising to a dangerous point: as already explained, the air stream partly serves this purpose.

Figs. 32 and 33, taken from Otto Durst's work (*Presshefefabrikation*), show the daily fluctuations in the amount of yeast and spirit produced during the months of January and July. From Fig. 32 it

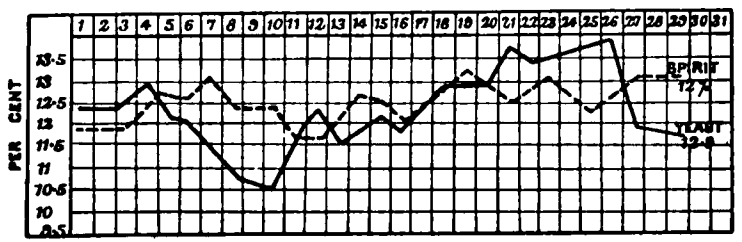

YIELD OF YEAST & SPIRIT. MONTH OF JANUARY.
PER CENT
SPIRIT 12 %
YEAST 12·8
(AFTER OTTO DURST)
MATERIALS:- MALT 27%. RYE 60%. BARLEY 5%. BUCKWHEAT 8 %.
MASH:-2 HOURS STAMO. TEMP 148 - 146 °F. PRELIMINARY MASH (VORMAISCH)
SOURED 14 HOURS. SULPHURIC ACID USED IN MAIN WORTS.

FIG. 32.

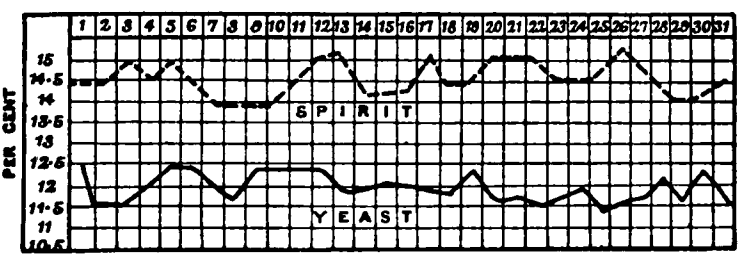

YIELD OF YEAST & SPIRIT. MONTH OF JULY.
PER CENT
SPIRIT
YEAST
(AFTER OTTO DURST)
MATERIALS:- MALT 27%, RYE 51%, MAIZE 16%, BUCKWHEAT 8 %.
SPENT WASH (SCHLEMPE) EMPLOYED. MASH TEMP 144·8 F.

FIG. 33.

appears that an average yield of some 12 per cent. or so of spirit was obtained, whilst the percentage of yeast on the materials averages

about 13 per cent. The yeast yield is low, owing probably to the fairly large percentage of unmalted cereals and consequent lack of yeast nutriment, and the fact that the worts were probably not aerated, for it has been found possible in pressed yeast manufactories, under the most favourable conditions, to obtain a yield of pressed yeast close on 25 per cent. by weight of the materials employed. The multiplication of the yeast depends on many influencing factors, e. g. the saccharine and nitrogenous richness of the worts, and the ratio of the one set of components to the other; the power of the yeast in the direction of multiplication; the acidity of the worts and the degree of aeration.

Hayduck's considerations relating to the effect of nitrogenous nourishment on yeast are of the first importance in relation to the question of yeast increase and alcohol production, and Dr. A. L. Stern's recent researches in the same field (vide Appendix) have also a very definite application to distillery practice. At the risk of repeating what has already been given, we may well give Dr. Sykes'[1] summary of the main conclusions to which Hayduck's experiments led. They are as follows:—The proportion of nitrogen in the dry substance of yeast varies from 3·92 to 10·30 per cent. Highly nitrogenous yeast multiplies more rapidly in a wort poor in nitrogen; on the other hand, yeast poor in nitrogen reproduces itself more rapidly in a highly nitrogenous wort. Hayduck found it possible to artificially vary the nitrogen content of a yeast by using a saccharine solution with nitrogen added in the form of asparagin, with suitable mineral nourishment; the quantity of asparagin being made variable. Hayduck found that in worts containing but small quantities of nitrogen, yeasts are produced with small but constant quantities of nitrogen. As the amount of nitrogen in the worts increases, so also increases in quantity the nitrogen of the yeast produced, to an extent fairly proportionate to the amount of nitrogen in the wort. Up to 0·2 gram of nitrogen per litre there is an increased yeast production, but beyond this the amounts of yeast produced are only very slightly greater. The nitrogen of the yeast still increases till one arrives at 0·5 gram of nitrogen in the wort, beyond which there is neither increased reproduction nor increased assimilation of nitrogen. Thus, by growing yeast in a highly nitrogenous wort it is possible to increase its percentage of nitrogen very materially; and the converse holds good, for if a highly nitrogenous

[1] 'The Processes of Mashing and Fermenting from the Distiller's point of view,' Walter J. Sykes, Ph.D., *Journ. Instit. of Brewing*, vol. v. p. 180 et seq.

yeast be fermented in a sugar solution simply, the percentage of nitrogen will fall perceptibly. In fermentations conducted at low temperatures in worts containing all the needed kinds of nutriment, the tendency is for the yeast to assimilate more nitrogen; in fermentations carried out at higher temperatures to assimilate less.

There are a few points left which seem of sufficient importance to merit dwelling upon.

In Continental distilleries a good deal of use is made of the spent wash remaining after distillation. This liquid, objectionable as it is to sight and smell, contains, nevertheless, some useful matters. It is rich in free acid—mainly lactic acid—and in phosphates: it also contains residual nitrogenous matters, which are of some service for yeast nourishment. It is generally employed to replace a portion of the mashing liquor of the chief mash, or of the preliminary mash—the Hefegut—but more often added to cool down the mash. It is most useful when the free acid is lactic, but if other fatty acids, e. g. butyric, be present in quantity it is likely to do more harm than good. In Germany, a good deal of the spent wash is concentrated and mixed with other matters, grains, &c., as feeding material for cattle. In this country it more often provides material for experiments on the treatment of sewage, this being the value which is commonly placed upon it. There are two developments likely to result from the influence of recent scientific research and discovery. The first is that Effront has succeeded in so modifying the normal action of certain races of beer yeast, by cultivating them in solutions containing fermentable sugar, potassium nitrate, and aldehyde, that they will more readily ferment the degradable dextrins. The second is the adaptation of an organism, Amylomyces Rouxii, discovered by Calmette in Chinese yeast, which can convert starchy matters into sugar, the sugar so produced being fermented by yeast which is also added. The two organisms grow together. A yield of 97·5 per cent. of the theoretical amount of alcohol is said to be obtained: the process is known as the amylo process. One of the great advantages is that diastase is not needed in the fermenting vessel, and consequently the wort can be boiled and thus effectively sterilized [1].

[1] *Journ. Fed. Insts. Brewing*, 1896, pp. 477-8.

CHAPTER XII

PURE YEAST CULTIVATION IN THE LABORATORY AND ON THE INDUSTRIAL SCALE. SKETCH OF THE LAGER-BEER PROCESS.

WE have already paid some attention to the subject of the varieties of Saccharomyces Cerevisiae used respectively in the breweries of the United Kingdom and in the Continental Lager-beer process. Two such distinct methods of brewing afford us two main groups of Saccharomyces Cerevisiae, but each of these groups is susceptible of differentiation to the following extent:—(1) As regards the sub-varieties of Saccharomyces Cerevisiae itself, which may be present in any given brewery yeast; (2) as regards the well-nigh unavoidable presence—on ordinary practical lines—of the secondary or wild yeasts. Owing to the comparative uniformity of the methods by which Lager-beer is produced, the differentiation into sub-varieties is doubtless less than in the United Kingdom, where, as we have pointed out, the variation in the methods of procedure—particularly as regards the fermentation—is so much more marked.

Before we can clearly follow the somewhat recent developments included in the term 'pure cultivated yeast,' it is desirable that a bird's-eye view should be taken of the ordinary Lager-beer process, noting the chief points in which it differs from the method pursued in England:—

1. The malts for Lager-beer brewing are less fully grown up on the malting floors, being ready for the kiln in two or three days less than in England.

2. The malt is kept for a less time on the kiln—sometimes two days less—and is dried off very pale (excepting in occasional cases where dark-coloured beers are brewed). These malts give very pale worts; they are more diastatic than English malts.

3. Double kilns are frequently used, the green upper 'piece' replacing the cured piece when the latter is unloaded from the lower kiln.

4. The malts are mashed on the decoction system, i. e. successive portions of the thick mash are withdrawn from the mash-tun, boiled in the mash-copper, and returned to the mash till the temperature of the whole is brought up to 30°–40° F. from the original initial heat. The result of this is to give a wort which contains the starch transformation products in the shape of maltose and degradable malto-dextrin or intermediate dextrins, with a smaller percentage of stable dextrin than is furnished by the English infusion system. Such a wort attenuates to a fairly low point, and goes on slowly fermenting long after the primary fermentation is over.

5. The boiling and hopping are very much on the same plan as in this country. Very good hops are employed in the proportion of about 6 lb. per quarter of malt for the lighter 'running' ales, 'Schank-bier' or 'Bock-bier,' and 8–9 lb. for Store or Lager-beer. The specific gravity of the worts averages about 1052·5, and the worts are cooled to 40°–50° F. for fermentation, according to the class of ale. The fermentation proceeds at a low temperature, and lasts from eight to fourteen days. A very moderate head is formed, appearing first as a slight froth, then becoming crinkled; very little yeast is found in the head, it is either in suspension during the period of activity of the fermentation, or else on the bottom of the fermenting vessel [hence the names it has acquired of 'sedimentary,' 'low,' or 'bottom' yeast, as distinguished from English 'top' or 'high' yeast]; the temperature of the fermentation rises a few degrees.

6. Lager-beer is usually racked from the fermenting vessel into capacious casks containing some twenty-five barrels apiece, which are stored in a cellar, the temperature of which is kept pretty near the freezing-point. Three months is about the time required for ripening Lager-beer: it is then racked into smaller sale casks, or bottled. Beech shavings are used in the Lager casks to assist the fining of the beer.

In the case of bottled beer—especially for export purposes—the process of Pasteurizing or sterilizing in bottle in much employed abroad. The method consists essentially in submitting the beer in bottle to a temperature of about 130° F. for half an hour or so by immersing them in a suitable bath. This treatment secures practical immunity from change within a moderate period—say a few months—most of the organisms being killed. It should be borne in mind that the low temperature at which the Lager-beer has been matured enables it to hold enough carbonic acid gas in solution to give what is called

'condition,' and therefore a secondary fermentation in bottle is not needed. Pasteurizing is not applicable to English beer bottled on the old system, but with the newer methods of cooling to a very low temperature, charging with carbonic acid gas under pressure and filtering brilliant through specially devised beer filters, it is a possible sequel, and the process has very considerable advantages greatly outweighing the slight drawback that the heating contributes a slightly burnt flavour to the beer.

The essential point of difference between the English and the foreign system of brewing, as regards the racial peculiarities of the ferment, lies in the range of temperature through which the fermentation is carried. The 'low' yeast does its work—much more slowly it must be confessed—at a range of temperature 20° F. below the average range in the United Kingdom.

An interesting recent development of the Lager-beer process, but one which has as yet by no means largely replaced the old methods, is the method of fermenting the worts in closed vessels under greatly reduced pressure: this is the so-called vacuum system. At the low pressure, fermentation is greatly accelerated, and in the vacuum cylinders it can be carried on under conditions of greater cleanliness and more uniformity as regards aeration, temperature, &c., than on the old plan. A few weeks, by this method, suffice to bring about a degree of attenuation and a general 'condition' which previously required as many months, and the beers so produced are very pleasant drinking; but so far as the writer's experience of them goes, they have a character of their own quite different from the Lager-beer brewed on the true Lager system.

Owing to the low-dried malts, the use of softer water than is used in the United Kingdom, and, doubtless, the somewhat lingering fermentation which does not cleanse the beers so effectually; added to the fact that the yeast performs its functions best at a low temperature, Lager-beer is rather subject to rapid deterioration if exposed to ordinary atmospheric temperatures—particularly, as may be supposed, in the summer months. Even the Pasteurized beers are not always immune, and sarcina and other bacteria not unfrequently play a part in destructive changes in them. It may, on the whole, be affirmed that Lager-beer is more prone to adverse changes from bacteria and wild yeast than English beer under similar conditions of storage; this is probably the main reason why so much attention has been bestowed abroad upon the biological side of the science of fermenta-

tion. Whether this is the cause, or whether it has been due to a direct scientific stimulus, the results of the various researches which we will presently outline, have been, and are of the very first importance.

We have just been speaking about the view that may be taken as to British and Continental brewing yeast forming two chief varieties of Saccharomyces Cerevisiae. This is true so far as it goes, but the fact is that in both cases we have normally to deal with rather a heterogeneous association of ferments. It is not only a question of varieties, but even of species. The consideration as to whether, for instance, 'skimming,' cleansing, and 'stone square' yeasts owe their typical action to the fact of being distinct varieties of S. Cerevisiae, sinks into the background when we come to take any one of these yeasts, and find that it is in most cases separable into distinct species. Some one variety of S. Cerevisiae may, it is true, be the predominant form present, and usually is so in a really typical yeast, but at the same time, other species of yeast may constitute a perceptible percentage of the cells present; until such time, however, as the percentage becomes very marked in quantity, any influence the foreign cells may tend to exert is suppressed in a vigorous primary fermentation, but may display itself very obviously in the secondary fermentation, especially if the composition of the wort should favour a free growth. We have already spoken of such admixture of alien species of saccharomyces in pitching yeast, as *wild yeast* contamination, and it is a very convenient mode of expression. Pasteur, in his researches on wine and beer, more than implied that foreign or wild yeasts might be a source of trouble in a fermented liquid, but his attention was being more particularly claimed by another class of organisms associated with fermentation, viz. bacteria, or, as he terms them, 'disease ferments,' and it was most natural that he should deal with these first, as the unscientific methods pursued by wine producers and brewers at that period had led to great losses by bacterial deterioration, in so many cases the result of uncleanly methods. The researches just alluded to were probably attended by practical results of far greater value than if the great scientist had taken up the more minute study of the life-history of the different species of yeast he encountered; for he was led to impress upon brewers the necessity for various reforms in the process, and to stimulate inquiry in a way which has in itself been most salutary. To Hansen belongs the distinction of establishing the fact that practical freedom of yeast from bacteria

does not imply freedom from abnormal secondary fermentation, and, acting on this discovery, he elaborated very complete and most ingenious methods for the separation of the species and well-marked varieties in any given specimen of yeast, studying the properties of each, and determining which were favourable in their action, and which were the reverse. The importance of these researches in their immediate and general outcome cannot easily be over-estimated, and it will be profitable to trace the steps by which Hansen arrived at pure cultivation of yeast, first on the experimental and then on the practical scale. Hansen's first important publication on this subject appeared in the *Carlsberg Reports* in 1879. It dealt with the organisms found in the air of Carlsberg and the neighbourhood, at different times of the year. Hansen exposed flasks of sterilized beer wort to the air, and allowed them to become infected with such organisms as happened to come into them, and then, after an interval, the flasks were examined microscopically to see what organisms were present. As a result, some twelve or fourteen species of mould were identified, amongst them—Penicillium Glaucum, Eurotium Aspergillus Glaucus, and Oidium Lactis, and a few unclassified moulds and some torulae. Twelve kinds of bacteria were distinguished, the commonest being Bacillus Subtilis (probably Van Laer's Saccharobacillus), Mycoderma Aceti, and Sarcina: last, but not least from our view of the matter, were seven kinds of Saccharomyces, as follow:—

S. Cerevisiae,
S. Ellipsoideus,
S. Exiguus,
S. Pastorianus,
S. Mycoderma (Mycoderma Vini),
S. Apiculatus,
S. Glutinis.

Subsequently, in 1882, Hansen applied this method of testing to the brewery buildings, and found that some parts contained many more organisms than others. He made the interesting discoveries, too, that an elevated temperature favoured the production of organisms in the flasks exposed, and that some of the organisms appeared even at 42°C. (107·6° F.), but such an elevated temperature favoured Mycoderma Vini more especially. Bacteria were particularly favoured by a temperature of 26°C. (78·8°F.). A very direct practical application of this knowledge was made by washing and purifying the air entering the fermenting cellars of the old Carlsberg brewery.

Some of the Saccharomycetes enumerated above were studied separately; amongst these was Saccharomyces Apiculatus: we have already alluded to the main facts elicited in this particular research.

Certain occurrences in the Carlsberg and other Danish breweries induced Hansen to experiment with a variety of S. Pastorianus obtained from some of the aerially infected flasks. On growing the particular ferment on a larger scale in beer wort, he found that the resulting beer had always a characteristic odour and a disagreeable bitter taste: brewings carried on side by side with S. Cerevisiae gave a normal beer from the same wort. Hansen justly concluded that this form of S. Pastorianus was a fruitful source of trouble on the practical scale, and as associated with a variety of S. Ellipsoideus was a positive source of trouble in the case of the Tuborg and Alt Carlsberg breweries. From the impure yeast of Alt Carlsberg four kinds of Saccharomyces were separated, of which only one—termed by Hansen Carlsberg low yeast No. 1—gave a normal beer: of the other forms the one designated S. Pastorianus I was the chief cause of mischief. The practical value of these results was at once apparent, and by 1884 yeast selection on a practical scale was an accomplished fact.

We may now very properly consider some of the leading points of the methods by which these results were arrived at. It may be said that from the very first the problem was to secure a growth of yeast known to proceed from a single cell, whatever the variety or species. Some useful pioneer work in the way of cultivation of organisms had been previously done by Nägeli, Lister, Klebs, Koch, and others, by arriving at an estimate of the number of bacteria contained in a given quantity of an infected liquid, and then diluting the liquid to an extent necessary to give, by calculation, an individual bacterium in a definite small volume—say 1 c.c. or a measurable fraction of this quantity. The difficulty, however, was to ascertain beyond a doubt that the observed growth following the purposeful infection of a nutrient liquid proceeded from one germ alone. This difficulty has been overcome in the case of both bacteria and Saccharomyces by so adjusting the nature of the nutrient to the organism under observation as to obtain colonies by the reproduction of an organism, lodged either in nutrient jelly, or attached to the walls of a flask containing some suitable liquid. This latter kind of cultivation is the one Hansen first employed for yeast; it is hardly applicable to bacteria, as they do

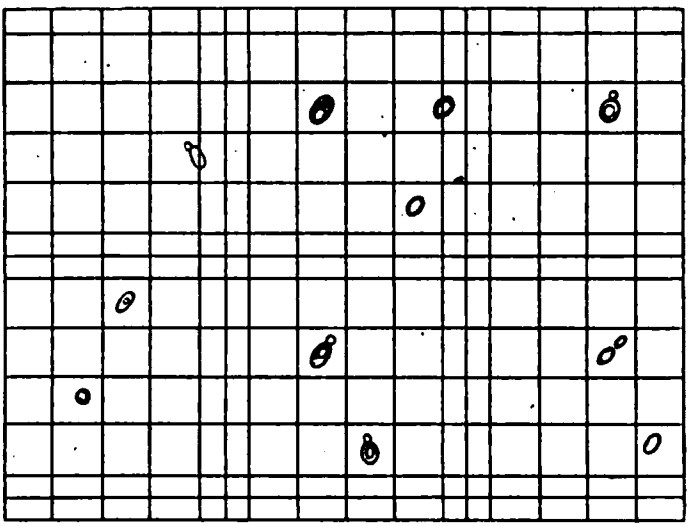
not as a rule attach themselves in the same way, but are at large in the liquid.

An instrument which has proved wellnigh indispensable for this kind of work is the so-called Haematimeter devised by Professor Panum of Copenhagen. It was originally designed for counting blood-corpuscles, but can be used to count many kinds of minute organisms if desired. The little instrument consists of a glass slide or slip about 3 in. × 1 in. In the centre a shallow cell or cavity is ground out, having a depth of 0·1 millimetre. In the centre of the cell two sets of very fine ruled lines cross each other at right angles, and in such a manner as to give a large number of minute squares having a side of 0·0025 mm. The cubical capacity of each cell then equals (0·0025 × 0·1) = 0·00025 c.mm. For convenience in counting the ruling is adjusted to give groups of sixteen squares (Fig. 34), and Hansen recommends that forty-eight to sixty-four squares and the cells contained in them be counted, so as to obtain a proper average of the number of cells per one square. The practical procedure is as follows:—A small quantity of yeast, say 1 gram, is mixed up and diluted in sterilized distilled water till it is faintly opalescent; this will serve as a first dilution, a drop being put in the Haematimeter and the cover-glass placed in position. This dilution may show perhaps an average of one cell per small square, i. e. one cell per ·00025 c.mm., which is equal to four million cells per cubic centimetre. If a dilution were now required containing, say, one cell in 1·0 c.c., it would be necessary to dilute the first dilution, say, as follows:—1·0 c.c. of the first dilution in one litre gives 4000 cells in 1 c.c., and if of this second dilution a third dilution of 0·1 c.c. in 400 c.c. of sterilized water be made, this ultimate dilution contains one cell per cubic centimetre. The second and third dilutions may each be examined and checked by the Haematimeter.

It will be discerned, almost without saying it, that the object is to bring about a state of dilution in which there is a reasonable certainty that a definite volume contains a definite number of cells, and that if a given volume of the dilution be used to infect, say, a number of flasks of wort, tubes of nutrient wort gelatine, or drops of gelatine, one should know within reasonable limits whether, say, one or twenty cells are introduced into the culture medium. The immediate question that arises is, What are the different modes of procedure likely to lead to successful culture? We have choice of the following:—

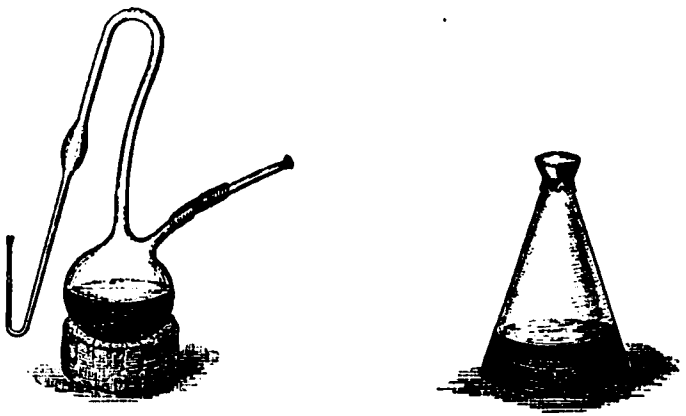
1. The small volume of infecting liquid can be introduced into

FIG. 34



HÆMATIMETER SQUARES WITH YEAST CELLS
× ABOUT $\frac{300}{1}$

a nutrient liquid such as beer wort, which has been filtered brilliant and sterilized by boiling in flasks and plugging with sterilized cotton wool (Fig. 35). If the endeavour has been to introduce one cell of



PASTEUR FLASK. ASSAY FLASK

FIG. 35.

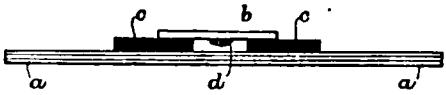
some Saccharomyces into a flask, it is better to inoculate at least six flasks, as it is probable that in one or two cases no cell has been introduced and the flasks will remain unaffected. It will be readily understood that even with the utmost painstaking, the dispersion of the yeast cells in the dilutions is not, and cannot be, perfectly uniform.

2. A somewhat preferable method to the above is to disperse a small known volume of the final yeast dilution in from 5 to 10 c.c. of sterilized nutrient jelly (see Appendix) which has been remelted at a gentle heat in the test-tube which contains it. The whole is poured whilst liquid into a sterilized Petri dish; if this is now kept at an ordinary sitting-room temperature, minute whitish-yellow spherules will be perceived after the lapse of a few days, and these spherules quickly enlarge into visible colonies. Any one of these colonies can be picked out by a sterilized platinum wire loop, and used as a means of inoculating a flask containing sterilized beer wort, or other nutrient liquid.

A modification of this method, which works very well, is to have a thin layer of nutrient gelatine already sterilized and solidified in a Petri dish, then to take, say, 0·5 c.c. of a dilution of yeast containing some 50 cells per cubic centimetre, and quickly but carefully brush it

over the set gelatine. The cover of the Petri dish is then replaced, and the dish is put into an incubator or kept in a not too warm place. Colonies appear on the surface of the gelatine after a few days, and can be picked off with a sterilized platinum wire or glass thread and reset.

3. A method which has found much favour, inasmuch as it enables the growing cell or cells to be kept under observation by the microscope, is the hanging drop or droplet culture. A good way of working this method is to take an ordinary glass slip (*aa*) (Fig. 36), and place on it a glass ring 1 or 2 mm. deep (*cc*); the ring may very well be divided from the glass slip by a ring of thin filter paper corresponding to the glass ring; the paper and ring being in position, the former is wetted and the ring pressed down somewhat firmly upon it; both the upper and lower surfaces of the ring are ground. Now apply some melted sterilized nutrient gelatine to one side of a thin cover-glass (*b*), and place this on the surface of the ring, which may be lightly vaselined, the drop of gelatine (*d*) being on the lower side of the cover-glass. When the gelatine is set, a drop of the yeast dilution is brushed on to the surface of the jelly on the cover-glass, which is replaced after the operation; any growth taking place in the gelatine can be observed by the microscope through the thin cover-glass. In order to keep the gelatine from getting too dry, moisture can be given to it by wetting the blotting-paper. When definite colonies have formed they can—as in the case of the Petri dishes—be picked off and reset. Another way of doing this is to transfer small portions of gelatine containing a yeast cell or two from a Petri-dish culture to the under side of the cover-glass, and then to allow the growth to proceed. For anything like systematic research or experiment of the kind just described, a rather considerable array of apparatus is needed; at the same time much may be done with ordinary laboratory appliances, and there is great scope for ingenuity in devising simpler apparatus to take the place of the very costly bacteriological appliances which are obtainable from many makers. The writer would suggest that the following items are wellnigh indispensable, but they need not entail very much



DROP CULTIVATION.
FIG. 36.

expense. One or two dozen litre and half-litre flasks, preferably of a conical shape (Fig. 35). A few dozen test-tubes of the ordinary size. A circular metal test-tube rack, ½ lb. of best sheet gelatine as near colourless as possible. Six inches of thin platinum wire, and some glass hairs or threads. Cotton wool.

Two or three Petri dishes.

One Haematimeter.

One or two hollow-ground slips.

Microscope slips with glass rings, filter-paper, and vaseline.

Some glass rod, and $\frac{3}{16}$ internal diam. glass tubing.

A water bath or saucepan into which the test-tube rack will fit.

A hot-water or jacketed funnel.

An incubator or thermostat. Klönne & Müller's is a very good and comparatively inexpensive one.

A gas regulator and burner for the incubator.

A hot-air sterilizing oven for test-tubes, flasks, &c., and a few smaller accessories of an inexpensive character.

A microscope is of course needed, and should be such as to give really good definition at a magnification of 300 to 400 diameters. One eye-piece and two objectives usually suffice for experimental work on yeast[1]; for bacteria a somewhat higher magnification is desirable, if not necessary.

Last, but not least, a quiet dust-free room of fairly equal temperature (preferably a north aspect) is required. Every effort should be made not to bring dust into the room nor to make it, for dust means aërial infection which may, if of a pronounced character, be very difficult to combat. If the room be not dust-free, a small operating chamber must be designed in the room in which manipulations, inoculations, &c., can be carried out without risk of aërial contamination: such chambers are employed in the Carlsberg Institute; they consist practically of a small glazed case or cupboard with a movable glass window-sash in front. The interior provides a few cubic feet of working space. The inner glass surfaces can be sterilized by sponging them over from time to time with a solution of mercuric chloride (1 gram per litre), a large stock of which may be kept. About 5 per cent. of glycerine may be added to the solution, which will then keep moist on the glass and catch up floating dust. A simpler form

[1] For further information on this point the student might consult *The Microscope in the Brewery and Malthouse*, by C. G. Matthews and F. E. Lott.

of the same apparatus is a small square glass-framed box, having two openings fitted with black calico sleeves for the arms of the operator. Elastic may be worked in round the openings so as to make the sleeves fit somewhat closely about the arms.

We are now in a position to follow Hansen's investigations with a clearer idea of what pure cultivation means so far as the laboratory operations are concerned.

At normal temperatures there are, it is true, fairly characteristic differences in form between many of the known species of Saccharomyces, and were these differences persistent under varying conditions of growth, there would not be very much difficulty in deciding, in a large number of cases, whether a given cultivation had proceeded, for instance, from S. Cerevisiae or S. Pastorianus. Hansen, however, soon discovered that the shape, size, and general appearance of cells, and the character of the fermentation they induced, did not suffice as a means of distinction ; for one and the same kind of Saccharomyces was found to be capable of exhibiting considerable variation in form corresponding to changes in the conditions attending growth, such as in temperature, plasma, oxygen supply, &c. As an example, a 'low' yeast growing with difficulty at 80° F. may give long branching cells, whilst at 45°–50° F. it would give the ordinary 'low' yeast forms. Not only was this a great difficulty to be faced, but Hansen found that even under fairly normal conditions he could obtain varieties of S. Pastorianus and S. Ellipsoideus, which did not differ very materially in appearance, but did differ very materially in function.

On the changes in appearance caused by variation of temperature, Hansen based the following method of distinguishing between different kinds of Saccharomycetes. He first made a microscopical examination of the sedimentary form of the yeast resulting from a completed fermentation under normal conditions of temperature, and then reset the yeast, and allowed it to develop in a wort at a temperature of 78°–80° F. The results gave information which proved to be of value, and to this test Hansen very soon added a further means of identification, viz. by noting the conditions under which ascospores were formed. For this method of experimenting sterilized blocks of plaster of Paris were employed, on which the yeast was poured, and which were then put into shallow glass dishes containing a little water, and a glass cover put over the whole. It was found that sporulation proceeded very slowly at low temperatures, and became more rapid as the temperature rose, till a point was reached where the development

was restrained, and at a higher point ceascd entirely. The lowest temperature for the six kinds of yeast first experimented with was 33° F., the highest limit 99·5° F., and between these limits there were characteristic points marking optimum temperatures, which differed according to the particular yeast under observation. The results are concisely and conveniently expressed in the following table, taken from a paper read by Dr. G. H. Morris in 1887[1]. (The equivalents of the centigrade temperatures in degrees Fahrenheit have been added.)

TABLE OF ASCOSPORE FORMATION.

Temp. Fahr.	Temp. Cent.	S. Cerev. I. (Hansen)	S. Past. I. (Hansen)	S. Past. II. (Hansen)	S. Past. III. (Hansen)	S. Ellips. I. (Hansen)	S. Ellips. II. (Hansen)
99·5	37·5	none	—	—	—	—	—
96·8–98·6	36–37	29 hours	—	—	—	—	—
95	35	25 hours	—	—	—	—	none
92·3	33·5	23 hours	—	—	—	none	31 hours
88·7	31·5	—	none	—	—	36 hours	23 hours
86	30	20 hours	30 hours	—	—	—	—
84·2	29	—	27 hours	none	none	23 hours	22 hours
81·5	27·5	—	24 hours	34 hours	35 hours	—	—
79·7	26·5	—	—	—	30 hours	—	—
77	25	23 hours	—	25 hours	28 hours	21 hours	27 hours
73·4	23	27 hours	26 hours	27 hours	—	—	—
71·6	22	—	—	—	29 hours	—	—
64·4	18	50 hours	35 hours	36 hours	44 hours	33 hours	42 hours
61·7	16·5	65 hours	—	—	53 hours	—	—
59	15	—	50 hours	48 hours	—	45 hours	—
51·8–53·6	11–12	10 days	—	77 hours	—	—	5·5 days
50	10	—	89 hours	—	7 days	4·5 days	—
47·3	8·5	none	5 days	—	9 days	—	9 days
44·6	7	—	7 days	7 days	—	11 days	—
37·4–39·2	3–4	—	14 days	17 days	none	none	none
32·9	0·5	—	none	none	—	—	—

It will be noticed that the maximum and minimum temperatures for the different species differ amongst themselves, as also the limits of temperature within which the ascospore formation takes place in the species mentioned. The differences are the most marked at the lower temperatures. By careful use of the foregoing data it has been found possible to discover the presence of $\frac{1}{100}$ of wild yeast in a sample of brewery pitching yeast. The wild yeasts sporulate with such com-

[1] 'The Pure Cultivation of Micro-Organisms with special reference to Yeast,' *Journ. Soc. Chem. Ind.*, 1887, p. 113 et seq.

parative ease that in most cases a cultivation on plaster would give a plain indication after forty hours at a normal temperature.

The problem of differentiating the species and varieties of Saccharomycetes in brewing yeast has been further dealt with by Hansen from the point of view of the varying conditions under which films or pellicles form on the surface of fermenting liquids. These films form at certain favouring temperatures, and generally appear towards the close of flask fermentations. Small flocks or islets of yeast come to the surface, and there develop a greyish-yellow slimy film, which if partially shaken down renews itself. A free quiet surface with direct admission of filtered air is essential to the growth. A conical flask covered with a filter-paper or loosely plugged with sterilized wool answers well. The points to be observed are:—

1. Temperature at which the film preferably forms.
2. Time elapsing before the film appears at different temperatures.
3. Microscopical appearance of the various films.

Plate VIII shows the sedimentary and film formations respectively of S. Cerevisiae I (Hansen) at different temperatures.

The following table, taken also from Dr. G. H. Morris's paper[1] just quoted, gives the facts connected with film formation in a very convenient and comprehensive manner:—

TABLE OF FILM FORMATION.

Temp. Fahr.	Temp. Cent.	S. Cerev. I. (Hansen)	S. Past. I. (Hansen)	S. Past. II. (Hansen)	S. Past. III. (Hansen)	S. Ellips. I. (Hansen)	S. Ellips. II (Hansen)
104	40	—	—	—	—	—	none
96·8–100·4	36–38	none	—	—	—	none	8–12 d.
91·4–93·2	33–34	9–18 d.	none	none	none	8–12 d.	3–4 d.
78·8–82·4	26–28	7–11 d.	7–10 d.	7–10 d.	7–10 d.	9–16 d.	4–5 d.
68–71·6	20–22	7–10 d.	8–15 d.	8–15 d.	9–12 d.	10–17 d	4–6 d.
55·4–59	13–15	15–30 d.	15–30 d.	10–25 d.	10–20 d.	15–30 d.	8–10 d.
42·8–44·6	6–7	2–3 m.	1–2 m	1–2 m.	1–2 m.	2–3 m.	1–2 m.
37·4–41	3–5	none	5–6 m.	5–6 m.	5–6 m.	none	5–6 m.
35·6–37·4	2–3	—	none	none	none	—	none

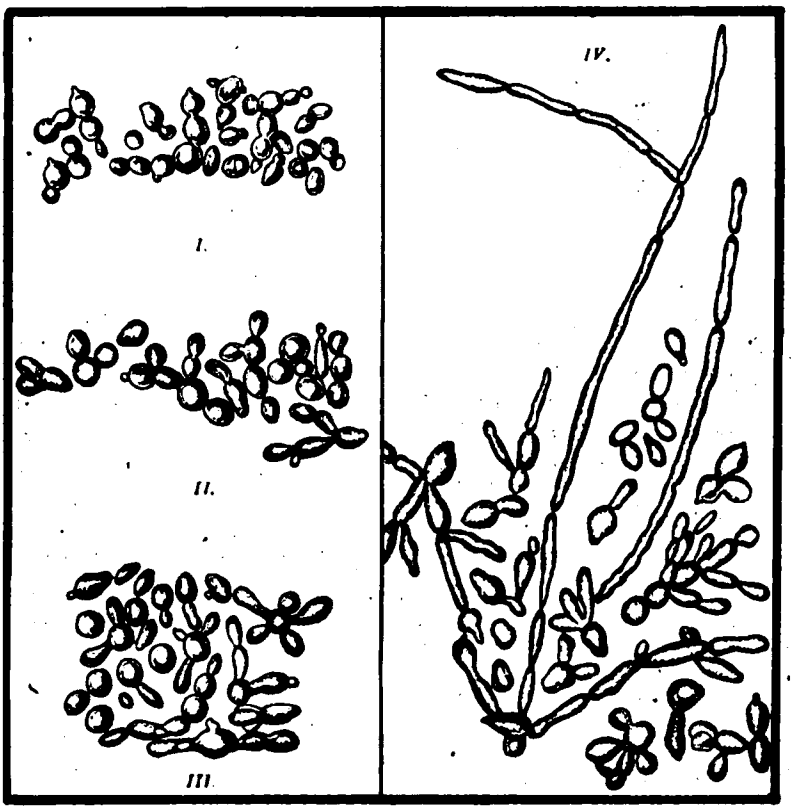
d. = days. m. = months.

By the conjunction of the foregoing methods of separation Hansen has positively identified the following yeasts.

S. Cerevisiae I. A 'high' yeast obtained from the pitching yeast

[1] *Journ. Soc. Chem. Ind.*, 1887, p. 113 et seq.

Plate VIII.



Growth of S. Cerevisiæ.

(*After Hansen.*) x 300.

I.—Sedimentary Form.
II.—Pellicle at 6-15° C.
III.—Pellicle at 20-34° C.
IV.—Pellicle (Old Culture).

of an Edinburgh brewery; afterwards found in London yeast. It develops ascospores between 11° C. and 37° C.: film formation at 13°–15° C.

S. Pastorianus I. Obtained from the air in the neighbourhood of the Carlsberg brewery, Copenhagen. It is a 'low' yeast, resembling that described by Pasteur. It imparts a bitter flavour to beer, develops ascospores at temperatures between 3° C. and 30·5° C.: film formation at 13°–15° C.

S. Pastorianus II. From the dust of the air. It is larger than Pasteur's or Reess's form. Acts mainly as a 'bottom' ferment. Causes no disease in beer. Develops ascospores between 3° C. and 28° C.: film formation at 13°–15° C.

S. Pastorianus III. From a 'low' fermentation beer produced in Copenhagen which was attacked by yeast turbidity (S. Ellipsoideus II proved to be present at the same time). It develops ascospores between 8·5° C. and 28° C.: film formation at 13°–15° C.

S. Ellipsoideus I. Obtained from the surface of ripe grapes from the Vosges. Effects on beer have not yet been investigated. It resembles the ferment described under the same name by Pasteur and by Reess. It develops ascospores between 7·5° C. and 31·5° C.: film formation at 13°–15° C.

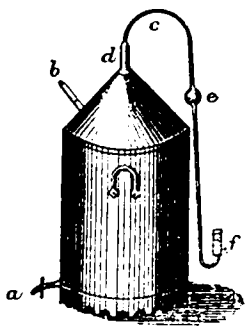
S. Ellipsoideus II. Associated with yeast turbidity in beer. Develops ascospores at temperatures between 8° C. and 34° C.: film formation at 13°–15° C. Very closely resembles S. Ellipsoideus I.

All these, with the exception of S. Cerevisiae I, may be regarded as wild yeasts, and their presence in appreciable quantity in a pitching yeast might, and probably would, entail a prejudicial effect on beer brewed with it.

The immediate practical results which Hansen obtained were, that he was able to select two varieties of 'low' yeast (one from the Carlsberg pitching yeast), pure-cultivate them, and introduce the pure cultivated yeasts into the Carlsberg and other breweries. No. 1 of these yeasts, obtained from the pitching yeast which had originally come from Munich and had been in use for many years in the Carlsberg brewery, gives a beer of great stability, and is very suitable for the old style of Lager-beer. No. 2 was isolated from Hamburg yeast, and was afterwards obtained from other sources. It gives a beer of less stability but more fullness or body.

So far we have dealt with the laboratory methods by which Hansen's epoch-making discoveries were worked out, but we should leave the

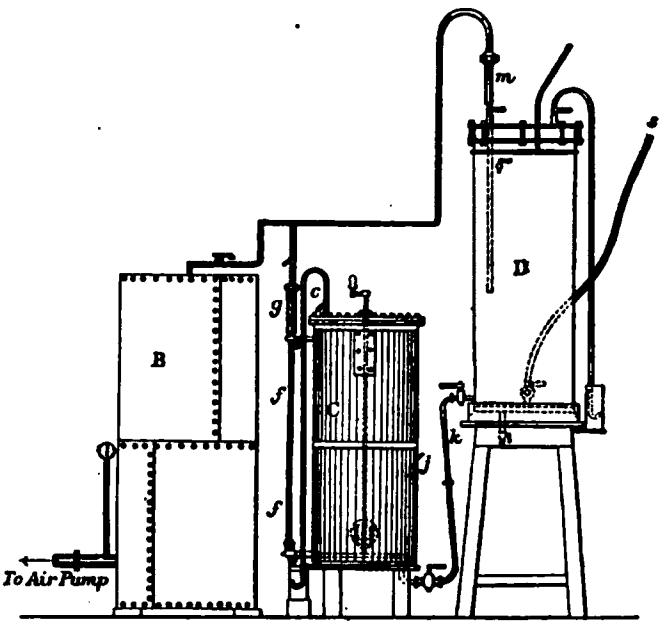
matter in a very incomplete state were we not to give at least a brief description of pure cultivation of yeast on the industrial scale. We may take up the matter at the point where an active, healthy fermentation has taken place in a litre-flask inoculated either with a single cell, or with a colony proceeding from a single cell separated and cultivated by one or other of the methods we have described. The yeast resulting from this experiment (after the fermented liquid has been poured away) or indeed the whole fermented contents of the flask, is introduced into what is called a 'Carlsberg vessel' (Fig. 37), a tinned copper vessel holding about two gallons. It is provided with an outlet tube *a*, an inlet tube *b*, a bent tube *c*, attached to the neck of the vessel *d*. The bent tube has a bulb-chamber *e* which is filled with cotton wool, and an air-filter *f* which consists of a small brass cylinder, which can be screwed on or off; it is filled with cotton wool or fibrous asbestos and provided with a loosely fitting cap.



CARLSBERG VESSEL.
FIG. 37.

The mode of procedure is as follows. The vessel is cleansed and partially filled with water which is well boiled in it by a large gas burner for one hour, the air-filter *f* and the stopper of the tubulure *b* having been removed. At the end of one hour re-stopper *b*, boil for a quarter of an hour more and allow the steam to pass through the bent tube *c*, also let a little boiling water run away at *a*, then re-stopper. Now take the source of heat away and put on the air-filter (*f*); run away the rest of the boiled water slowly at *a*, when filtered air will pass through *f* and replace the water. The vessel is now sterile and ready to receive beer wort. One and a half gallons of wort are introduced through *b*, the air-filter is removed, the wort well boiled, a little being run off at *a*. Towards the end of the boiling the bent tube is heated by passing the flame of a Bunsen burner along it. Take away the source of heat, put on the air-filter, which has been sterilized in a hot-air oven, and allow to cool; leave for several days to see if the wort is sterile, that is to say, that no turbidity from organisms or fermentation sets in, after which the products of a flask fermentation can be introduced at the tubulure *b*, and a not inconsiderable quantity of yeast be gathered from the completed fermentation. If necessary

four or five such vessels can be set with flask yeast at any given time. Inasmuch as the pitching of even twenty barrels of beer wort would require a gallon or two of thick liquid yeast, it is necessary to pitch the yeast from the Carlsberg vessels into still larger pieces of apparatus, so devised that fermentation can be carried out continuously without risk of aërial or other accidental contamination or infection. The first practical apparatus of this kind was devised by Hansen and Kühle. Modifications of this were made and used by Jörgensen and



HANSEN – KÜHLE APPARATUS.

FIG. 38.

Bergh, and in this country by Brown and Morris, and at the present time there are doubtless plenty of patterns, but the underlying methods and principles are pretty much the same. The Hansen-Kühle apparatus (Fig. 38) consists of three parts connected together by tubes. An air-pump *A* is used to feed a reservoir *B*, the latter being able to discharge air at pressure into the wort-cylinder *D*, and into the fermenting cylinder *C*. (These vessels are shown in section in

the illustration.) A small engine works the air-pump, thus compressing air in *B* at a pressure of a few atmospheres. The wort-cylinder *D* is sterilized by means of superheated steam, and is then filled with sterilized air from the air reservoir, purified by being filtered through cotton wool contained in a metallic vessel *m*. The wort-cylinder is then filled with boiling wort from the main supply from the copper (*S*). The wort is cooled by means of a system of tubes through which cold water circulates, and the necessary amount of air for aeration is allowed to enter through the filter *m*.

The fermenting cylinder *C* is sterilized in the same manner as the wort-cylinder. It is provided with a filter *g* similar to that in *D*; a glass tube *ff*, through which can be seen the level of the liquid; a suitable exit *c*, through which the evolved carbonic acid gas can escape; a stirring apparatus *b*, used to mix the yeast with the liquid; and a small tube *l*, through which the yeast can be introduced and also small samples removed. The tap *j* for drawing off the fermented liquid is so constructed that no contamination from without can enter.

The wort is transferred from the wort-cylinder to the fermenting vessel by means of the tube *k*; pressure of air can be used to assist this operation. When the level of the wort reaches a certain height, namely, just below the opening *l* through which the yeast is added, the supply of wort is stopped until the yeast has been introduced. Wort is then run continuously in until some 48 gallons are contained in *C*. If the fermenting vessel *C* be once seeded with pure yeast in this way, a continuous supply can be obtained for a twelvemonth or more. The fermenting vessel is provided with a rouser *b* to keep the yeast in suspension if necessary, to help to aerate, or to bring sedimentary yeast into a state of diffusion again after a period of quiescence.

The wort can be cooled in *D* to any desired temperature, and it is possible to arrange attemperators so that a control over the temperature can be exercised in *C* also. This latter vessel is generally lagged with wood, and it is better still if a non-conducting medium such as silicate wool, &c., be placed between the lagging and the metal, as fermentation can then rise in temperature by normal development of heat and pursue a natural course.

The fermentation is completed at the end of ten days, and the fermented liquid is then drawn off, and sterilized air allowed to enter at *g*. When the greater part of the fermented liquid is drawn off,

the tap is shut and fresh wort is run in from the wort-cylinder. The wort and yeast are well mixed, and about 5½ gallons of the mixture run off; this is repeated and another 5½ gallons of the second mixture withdrawn. These 11 gallons contain enough yeast to set 176 gallons of wort, which may be done in a specially devised fermenting vessel on the general plan of *C*; this next fermentation should give yeast enough to pitch the ordinary brewery fermenting vessels. The 11 gallons of yeast and wort having been withdrawn from the 'Hansen,' the fermenting cylinder is once more charged with wort from the wort-cylinder, and a fresh fermentation is inaugurated. One fermenting cylinder so used can be made to yield sufficient pure yeast to set 14½ barrels of wort each month. In working the apparatus the two chief points to be borne in mind are (1) to so completely clean the vessels that they are effectually sterilized; (2) to maintain an excess pressure of sterilized air during the cooling and withdrawal of the liquid. If these precautions are observed no contamination by impure air can take place.

As an indication of the scale on which pure cultivation was carried out even in the earlier days of this revolution in Continental brewery practice, it may be stated that Hansen, starting from the single selected cell, produced in a few weeks the whole bulk of yeast (5,500 lbs.) employed in the Alt-Carlsberg Brewery. For some ten or twelve years selected yeast has been in continual use in the Carlsberg breweries and in many other Continental breweries, and it may be said that, on the whole, the results have been extremely satisfactory, and that beer of more uniform character, higher standard of quality, and greater stability, have been brewed than could possibly have been produced on the old lines with a yeast of varying impurity. Still, there are some attendant disadvantages and sources of irregularity whose nature is but imperfectly understood; indeed, it would be strange if the process had not some imperfections when one considers that it is a living organism that has to be dealt with. The present state of the question may be described more or less in Dr. Heinrich Will's words: 'Despite the great advantages resulting from the use of pure cultivated yeast, abnormal phenomena have declared themselves which are not so much the result of the system as of variation in the management and conduct of the same. These adverse phenomena appear to have their origin in an imperfect state of knowledge respecting the conditions attending the nutrition of the yeast in the liquids in which it is destined to grow, and of the properties of the

specific yeast which is being used.' Notwithstanding the drawbacks, it is probable that not a single brewer who has used pure yeast would wish to revert to the old condition of things. Dr. Will says further that the chief cause of abnormal behaviour and deterioration of pure culture yeast is the lack of important nutrient matter, organic or inorganic. [It is more than probable that the improper balancing or ratio of the respective nutrients to each other is a factor of very great importance.] The visible signs of deterioration of pure yeast are:—failure of the yeast to attenuate the wort to the proper point; falling off in the crop; failure to settle at the close of fermentation; the production of unusual aroma. The question is, how far can the deteriorated yeast be restored? This question has been, in a measure, solved by selection from, or recultivation of, the pure yeast itself, for the very important fact has been discovered (falling under the 'law of variation') that the progeny of one cell may differ very materially in properties amongst themselves, and it is possible to select the cells which give a favourable result from those which give an inferior result, and so to start afresh from this point. The whole matter has plainly arrived at a complex phase, but one, nevertheless, which is calculated to stimulate anew the genius of such workers as Hansen and his colleagues.

As regards the adaptation of pure cultivated yeast to English methods of brewing, much may be said for and against; but on theoretical grounds, and so far as direct evidence is obtainable, it would seem that the peculiarities of the English high fermentation methods preclude the ready adaptation of the system. In the first place, British methods of brewing, where pursued with good materials, cleanliness, and technical skill, tend in the direction of producing a definite type of yeast corresponding to the particular system employed, and this fairly uniform type of yeast can be obtained, without any very special precautions, free from bacteria and containing a percentage of 'wild' or subsidiary yeast so small that it remains in abeyance during the primary fermentation, and only comes into action after the beer has been racked into cask, thus producing secondary fermentation or aiding in producing it.

Secondly, each distinct system of brewing requires a more or less distinct type of yeast to give the best results with the worts obtained and the mode of fermentation adopted.

Thirdly, whatever type of yeast is used, the beer must carry, after the primary fermentation is over, matters that are fermentable by

subsidiary or wild yeasts, otherwise it will remain flat and undrinkable; that is to say, if the primary fermentation has been completed. It is really this matter of secondary fermentation—which on the present lines of production is absolutely necessary for the conditioning of 'high' fermentation beers—that is the determining factor in the question of the adaptability of pure yeast to English brewing. No single cultivated yeast has as yet been found which will fulfil the required conditions, and composite yeasts (i. e. pure yeasts to which a certain proportion of a secondary or subsidiary yeast has been added) have not met with any lasting success. There has been no lack of experiments in this country on an industrial scale, for two or three brewing firms have worked for a period at least with pure cultivated yeast. In Burton-on-Trent, H. T. Brown and G. H. Morris gave much attention to the subject, and the latter of these gentlemen has recently published[1] the very instructive results which were obtained. The chief practical difficulty encountered was that the pure yeast seemed to possess an attenuative power which, beginning high, increased rapidly from brewing to brewing, and which, moreover, so exhausted the beer of fermentable matter that a very stubborn beer was produced, having little or no power of conditioning in cask, and requiring in all cases the addition of sugar solution ('priming') before it could be sent out for consumption. A kind of sick fret was also noticed in the flat beers, due, Dr. Morris surmises, to the presence of yeast-cell fluid in the beers. The beers were sound and, indeed, of a high order of stability, but very flat unless assisted in the manner described. Brown and Morris experimented on at least five kinds of yeast selected from different brewery yeasts, and in the publication just referred to the latter says that plenty of yeasts are to be found which will effect the primary fermentation and give perfectly palatable beers of good flavour, but they would remain absolutely flat, and in this state would be, of course, unsaleable. It would be possible, he believes, to induce secondary fermentation by adding another pure yeast, but it would be a rather cumbersome process, and he thinks that the advantages to be gained would not be counterbalanced by the difficulty in carrying it out. In this connexion we may say that Dr. Van Laer has produced so-called composite yeasts containing a primary and secondary ferment by synthetical methods, and doubt-

[1] 'Some Experiences in the use of Pure Cultivated Yeast,' G. H. Morris, *Journ. Fed. Insts. Brewing*, 1900, p. 333 et seq.

less with some measure of success as regards practical results; there is, however, no evidence which is at all convincing that these artificial mixtures retain the desirable characteristics for any length of time. The chief reason probably is that the ratio or proportion of the one kind of yeast to the other soon becomes disturbed, and this unsettling of the relationship which one Saccharomyces bears to the other is bound to have a corresponding effect in a direction which can be predicted by our knowledge of the functions of primary and secondary yeasts respectively.

Reverting once more to Lager-beer brewing, it is more than probable that the low temperature at which Lager-beer is fermented, and the quasi-sluggish nature of the fermentation, very much favour the use of pure cultivated yeast which, under the conditions obtaining, has not vigour enough to remove the whole of the fermentable matter which would disappear in a 'high' fermentation. This is equivalent to saying that the primary fermentation is incomplete, and this view quite accords with what Dr. Morris says as to Lager-beer from pure cultivated yeast containing, not only readily degradable malto-dextrin, but probably unfermented free maltose in addition.

The introduction of pure cultivated yeast has been a scientific step of the highest practical importance, and has led to a great increase in our knowledge of fermentation processes. Pure yeast has been used in connexion with wine fermentation and in the manufacture of cider, and the great pressed-yeast industry has profited very materially by the application of Hansen's discoveries. Without entering into detail, it can be easily understood from our knowledge of the varying functions of different species and varieties of Saccharomyces how, in the case of wines especially, the more desirable flavours and aromas can be obtained to the exclusion of the less desirable in fermented liquids, by employing the yeasts which selective experiment has demonstrated to be those best adapted for the particular end in view.

APPENDIX A

Appearance of Yeast Cells by reflected light

When yeast cells are examined in the ordinary way under the microscope, with the light from the source of illumination passing through them (transmitted light), what is seen by the eye is not the true appearance of the object as a whole: this last can, however, be obtained by cutting off the light that passes through the substage or diaphragm, and contriving that the slide of yeast under examination shall be illuminated from the side or from above, so that the cells reflect the light. If this is successfully arranged, the cells are seen to be spherical or oval bodies showing a beautiful pearly white or frosted appearance, the inner differentiation of the cell being almost invisible, but the surface very plainly discernible.

APPENDIX B

Dyes and Reagents in connexion with Yeast testing

Staining Fluids. A solution of methyl violet may be obtained by dissolving a few crystals of the dye (a small piece of violet copying-ink pencil will serve very well) in, say, 100 c.c. of distilled water. The solution should be quite transparent. If the solution be of the proper strength it will only stain the dead and torpid cells in a yeast sample. The simplest mode of applying the test to yeast is to replace the drop of water ordinarily used on the slip by a drop of the stain: stir the yeast into it till slightly milky, then put the cover-glass on.

Eosin may be used instead of methyl violet for yeast staining: it is also a very useful stain for bacteria. Ordinary red ink is now usually

made from eosin, and will, if necessary, serve as a supply—a few drops of the eosin ink in 100 c.c. of distilled water, carrying 5 or 10 c.c. of spirit of wine, will provide a suitable dyeing liquid; or a few crystals of eosin may be dissolved in 5 c.c. of alcohol and made up with distilled water to 100 c.c.

Iodine Solution. Add a few drops of tincture of iodine to, say, 100 c.c. of distilled water, carrying 5 or 10 c.c. of spirit of wine. Or 4 grains of iodine and 10 grains of iodide of potassium may be added to 100 c.c. of distilled water. The solution should be the colour of a full golden sherry. It may be used for testing yeast for glycogen (the cells containing glycogen colour a reddish brown); it will also tinge the dead and torpid cells a brownish shade, will stain starch granules blue, and will cause a sharper definition of any bacteria which may happen to be present with the yeast or the liquid under examination.

Magenta Solution (for yeast staining). 0·1 gram crystallized aniline magenta, dissolved in 160 c.c. of distilled water carrying 1 c.c. of spirit of wine, may be used instead of the methyl violet and eosin in experiments on yeast staining.

Ammonia Solution. A solution of ammonia is sometimes useful for dissolving away resinous particles (especially matter derived from hops) in 'fields' of yeast. About 10 c.c. of strong ammonia may be added to 100 c.c. of distilled water. A moderately strong solution of caustic soda or caustic potash serves the same purpose. 10 grams per 100 c.c. will suffice.

Noto. It is very convenient to keep a little wash-bottle for distilled water only amongst the accessories on the microscope table. The reagents, stains, &c., may be in ordinary stoppered bottles of convenient size, and used by means of small pipettes.

APPENDIX C

Testing the Fermentative Power of Yeast

Method I.

The author believes that the modification of the method of testing yeast to be described is properly attributable to J. Heron.

Into a flask holding about 250 c.c. is introduced 100 c.c. of a 5 per cent. solution (5 grams per 100 c.c.) of cane sugar. (It is advisable to use pure recrystallized sugar, of which a stock should be kept.) The temperature of the sugar solution should be 70° F. ¼ oz. avoir. of pressed yeast or ½ oz. of liquid yeast is then added, and the flask is corked and joined up to the rest of the apparatus shown in the figure. The flask must either stand *on* a copper vessel such as a forcing tray maintained at 70° F. or *in* a water-bath kept at this temperature (Fig. 39). By means of a small exhausting syringe suction is applied at P, and water is drawn up from the vessel V into the large measuring tube T. It is better if the water used has been previously saturated with carbonic acid gas. The measuring tube should have a total capacity of about 500 c.c. and be graduated in 10 c.c. intervals. The connexions being all sound, and sufficient water in V to leave the vessel one-third or half-full after T is filled by suction, the clip C is closed. Fermentation sets in very soon, and the carbon dioxide produced passes over into F. The experiment may be continued for six, nine, or twelve hours, but obviously every yeast tested should be given the same time. At the end of the period determined upon, the volume of gas is read off in T, a note made of the quantity, and the fermentative value of a given weight of yeast in a given time thereby determined. If the water be previously saturated with CO_2 a truer record of the gas evolved will be arrived at, as very little will be absorbed by the water; but if plain water from the same source be customarily used, the experiments will be comparable: either one condition or the other should be adhered to. If the yeast be for distillers' purposes it may be tried at 80° F. If for bakers' purposes, a paste of flour and water (vide next method) will give a more valuable indication.

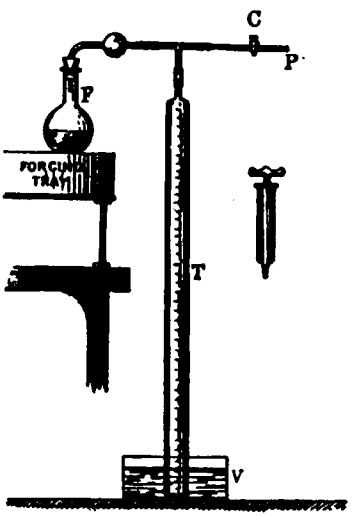


FIG. 39.

METHOD II.

The author has obtained very good results in the following way:—A very strong tinned copper vessel (Fig. 40) holding about six pints was made for the particular purpose, and was tested up to a pressure of 250 lb. per square inch. The vessel was fitted with a pressure gauge registering up to 180 lb. per square inch. The gauge screwed into a strong neck of about $\frac{1}{8}$ inch internal diameter, and either gutta-percha or lead washers were used to make a good joint. The vessel was fitted besides with a bib-cock at the bottom and a little screw manhole on the shoulder. Fermentations were first tried with beer wort set with yeast, and indicated pressure up to about 160 lb. was easily obtained.

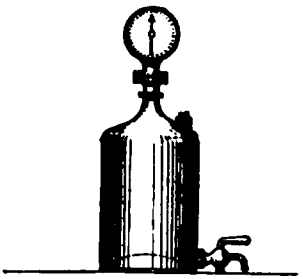


FIG. 40.

For a general method of yeast-testing the following was adopted. $4\frac{1}{2}$ ounces of large-crystal cane sugar were dissolved in water, and 1 ounce of the yeast in a pressed state added (liquid yeast was pressed in a cloth in a small hand-press) and the whole volume made up to 3 pints. The solution thus made had a specific gravity of about 1,029, and the limit of pressure producible from it was estimated experimentally to be about 100 lb. per square inch. The solution being gathered at 60° F. and the vessel closed up, the indicated pressure could be read off at intervals. The result could be accelerated by immersing the pressure vessel in a water-bath at 70° F. or even 80° F., the solution having been warmed to the same temperature before the addition of the yeast. The following were some of the results obtained (temperature 65° F.):—

Hours.	Pressure (A) lb. per sq. in.	Pressure (B) lb. per sq. in.
1	10	7·2
2	19	16·0
3	29	26
4	39	38·5
5	46	45
6	51	54
7	58	61
8	62	68

(A) A baker's yeast of fair quality.
(B) A brewer's yeast of good quality.

Now in this instance—as in many others—the brewer's yeast proved useless for baking purposes, and yet was a very vigorous ferment, for although somewhat slow in starting it practically drew level with A at the fourth hour. It became apparent from this and kindred results that the activity in respect of the fermentation of sugar was no criterion as to the baking power. The author, being thus led to seek some better way of estimating the relative powers of yeast samples from the baker's point of view, thought that it would be better to try the yeast with flour itself—first, however, gelatinizing it and cooling the thin paste to the determined temperature at which the yeast was added. 4½ ounces of wheat flour were stirred up with cold water, and into the creamy mass 2 pints of water at 180° F. were run, with thorough stirring, the mixture was cooled to 80° F., and the volume, after working in 1 ounce of pressed yeast, made up to 3 pints with water at 80° F. The charge being introduced into the vessel, the latter was closed up and placed in a water-bath maintained at 80° F. The following were some of the results obtained:—

Hours.	Pressure (A) lb. per sq. in.	Pressure (B) lb. per sq. in.
1	5	3
2	9	6
3	17	7·5
4	24	9·0
5	30	10·5
6	37	12·0
7	42	14·0

(A) A good French baker's yeast which made a very good loaf.
(B) A weak yeast which gave a very bad loaf.

We have thus methods which can be applied respectively for the determination of the fermentative power of brewers', distillers', or bakers' yeasts as the case may require. For the two former, testing with cane sugar suffices, but for bakers' purposes this is practically useless, for the kind of activity required in a baker's yeast is such that it must be able to act on soluble or gelatinized starch as well as attack ordinary fermentable sugars. Even with flour paste the results are occasionally anomalous, and the best test of all for a baker's yeast is to make and bake a small loaf of bread under standard conditions. Matthews and Lott have frequently used the following method, the baking being performed in a copper hot-air oven of sufficient size.

½ ounce of the yeast to be tested is taken (it must be in the form

of pressed yeast) and intimately mixed with 2 ounces of flour and 4 ounces of water at 80° F. This forms what is called the ferment, which is left covered over (a porcelain dish is very suitable with a dial glass over it) for two hours at 80° F. At the end of this period the 'ferment' is mixed with 6 ounces of flour and the resulting mass is thoroughly kneaded, placed preferably in a small oblong tin (a model of an ordinary bread tin), and left for one and a half hours in a place where the temperature is about 80° F. The hot-air oven being in the meanwhile heated to a temperature of about 300° C. (if 300° C. cannot be obtained, work at a constant temperature at some lower point), put the tin containing the dough in it and bake for twenty minutes (thirty minutes may be needed at a lower temperature). A good yeast should under these conditions give a good-looking light loaf, the top of which is well above the level of the tin. In whatever way the above conditions may be modified to suit circumstances, the chief point to observe is that they be kept constant so that different yeasts may be tested under the same conditions. One advantage of this method is that several yeasts can be tested simultaneously.

APPENDIX D

CONJUGATING YEAST[1]

A CONJUGATING yeast has been obtained from commercial ginger by B. J. P. Parker, and developed and studied by cultivation in gelatine. The yeast shows typical ovoid and spherical cells which may become sausage-shaped in old cultures. The yeast was particularly studied by means of hanging-drop cultivations at 25° C. The cells exhibited vacuoles and bright granules, and after a while from many of the cells proceeded a beak-like tubular process. The beaks or tubes of contiguous cells met and fused together, a transfusion of protoplasm taking place between the cells thus united. Formation of spores then ensued, a group of spores being formed surrounded by a membrane. The size of the ripe spores was from 4–5 μ.

The yeast ferments the ordinary sugars, dextrose, levulose, and

[1] *Brewing Trade Review*, 1901, p. 268. Abstracted from a paper communicated to the Royal Society by Professor Marshall Ward.

sucrose. Parker proposes that the yeast should be classified in the new genus according to Beijcrinck's suggestion—the Zygosaccharomyces.

APPENDIX E

THE NUTRITION OF YEAST[1]

A. L. STERN (*Journ. Chem. Soc.*, 1899, 201-206; 1901, 943-953, and *Journ. Fed. Insts. Brewing*, 1899, 399-407) has investigated the effect of varying conditions on yeast nutrition.

The fermentable solution employed contained potassium phosphate, magnesium and calcium sulphate, asparagin, and dextrose in definite proportions. The fermentation was started by seeding with a measured quantity of yeast obtained continuously in the same way from one stock, and carried on at one temperature throughout. Every detail was so arranged that the conditions of any one experiment could be exactly reproduced if necessary, this being essential for the purpose of the investigation. Under these conditions the weight of the yeast crop and the amount of nitrogen contained in it were less than when such nutriment as is contained in malt worts, yeast water, &c., is available for the yeast. It is therefore possible that the results obtained may be in some respects abnormal; this the author hopes to determine in the near future.

I. Inorganic Nutriment. It was known that potassium phosphate, and magnesium and calcium sulphate, could supply all the inorganic nutriment the yeast required; the presence of potassium and magnesium phosphate had been previously shown to be essential. It is now shown that the presence of sulphate in conjunction with the foregoing is also essential. The sulphate could be effectively replaced by thiosulphate or sulphur, but not by thio-carbamide, potassium sulphocyanide, or sulphonal.

So long as a sufficient amount of the inorganic nutriment was present to supply the maximum requirement of the yeast, any larger quantity had no effect on the resultant yeast crop.

[1] Kindly contributed by Dr. A. L. Stern at the author's invitation.

II. Nitrogenous Nourishment. Using asparagin as the source of nitrogen for yeast growth, it was found that so long as sufficient was present to supply the maximum yeast requirement, any excess led to no increase of yeast crop or of its nitrogen content. Other results obtained in this investigation are in accord with Hayduck's work.

Leucine, tyrosine, and urea were found to be capable of serving as nitrogenous yeast nourishment.

III. Carbohydrate Nutriment. Dextrose was the only carbohydrate experimented with. It was found that, as the concentration of the sugar was increased, the weight of the yeast crop obtained on the completion of the fermentation, as also the nitrogen contained in the crop, increased; the rate of increase of both crop and nitrogen for the same increase in the sugar concentration being greatest in the weak sugar solutions, and gradually falling off as the strength of the solution was raised.

IV. Reduction of Sulphates, &c. When asparagin was employed as the nitrogenous nutriment, it was found that the gases evolved during the fermentation, from which the above results were deduced, smelt of sulphuretted hydrogen. When some other source of yeast nutriment such as leucine, tyrosine, or urea was employed this was not the case. It appears then that, under certain conditions, the production of stench during fermentation may be due to the reduction of sulphates by yeast [in presence of certain forms of nitrogenous aliment.—C. G. M.].

V. Influence of Temperature on Nutrition. Fermentations carried on at temperatures between 12° C. and 25° C. were found on completion to yield approximately the same weight of yeast with the same content of nitrogen. Fermentations at higher temperatures resulted in a weakening of the reproductive functions of the yeast.

VI. Influence of the Amount of Seed Yeast. It was found that if a weight of seed yeast A be added to a fermentable solution, on the completion of the fermentation the weight of the yeast crop will be A + B, where B is dependent on the composition of the fermentable solution. [It is plain that for there to be any positive value for B, the seed yeast must be in less quantity than the non-multiplying amount (Adrian Brown's experiments). It is also possible, under certain peculiar conditions where excess of yeast is used and a very quick fermentation promoted—with or without aeration—to get less yeast by weight in the crop than was added for pitching. This is due to the cells parting

with cell contents during the stress of fermentation.—C. G. M.] The same relation holds good between the weight of nitrogen contained in the seed yeast and the yeast crop. Whilst these experiments show that the weight of the yeast crop is dependent on two factors, viz. the weight of the seed yeast and the composition of the solution, A. J. Brown showed that the number of cells in the yeast crop was dependent solely on the composition of the fermentable solution [? if the seed yeast added was under the non-multiplying limit.—C. G. M.].

VII. Yeast Growth at Different Stages of the Fermentation. Previous workers have stated that most of the yeast is formed during the early stages of the fermentation, and that during the later stages little if any yeast growth takes place. This was, however, not found to be the case in these experiments. The growth of yeast was found to go on until the fermentation was completed, and a definite relation between the weight of yeast formed and the amount of fermentation was noticed, dependent on the amount of seed yeast employed (a curve showing this relationship in a graphic form will be found on page 501 of the *Journ. Chem. Soc.* for 1901).

The experiments indicate that, whilst the function of the nitrogenous and inorganic nutriments is solely to supply the formative material for the yeast, the function of the sugar is something more than this. The energy liberated by the relatively large weight of sugar decomposed into alcohol and carbon-dioxide no doubt appears chiefly as heat, raising the temperature of the fermenting solution and thus aiding fermentation, but the published measurements of the heat evolved indicate the existence of a balance between these results and the calculated amount of energy liberated by the decomposition which may very well be employed by the yeast for the performance of its vital functions.

Whilst these experiments indicate the close connexion between fermentation of the sugar and yeast growth, yet the existence of a fermentative enzyme apart from the living cell cannot be considered to be disproved by them. The known facts indicate that the enzyme is not contained in yeast that has remained quiescent for any length of time, but must be abundantly secreted by yeast as soon as it is introduced into a fermentable solution. The change in appearance which yeast undergoes under these circumstances, the author points out, is similar to the change in appearance of the columnar cells of the barley embryo when secreting diastase.

APPENDIX F

Combined Action of Diastase and Yeast on Starch Granules [1]

It has been known for a considerable time that when starch conversions containing the so-called stable dextrin are acted on by yeast in the presence of diastase, the action is greatly in excess of that produced by either yeast or diastase acting alone; in fact the so-called stable dextrin which is not acted on either by yeast or by diastase under ordinary conditions, may be completely fermented by yeast in the presence of active diastase. Dr. G. H. Morris now shows that ungelatinized starch granules submitted to the simultaneous action of malt extract and yeast may have three times as much matter dissolved from them as by malt extract alone. The enhanced action is not due to the removal of the maltose by fermentation. If the fermentation be arrested by addition of chloroform no increased action of the malt extract is observed, nor does any increased action take place when the cold-water malt extract is first fermented and then the yeast cells removed prior to adding the liquid to the starch granules. The joint action only occurs with starches, such as barley and malt starch, which are susceptible of being attacked by diastase in the ungelatinized form. Potato starch in the granular state is one of those that does not respond to diastase, and remains untouched by malt extract and yeast acting together.

Dr. Morris remarks that the above results are especially interesting in view of the attention which has of late been given to instances of so-called symbiotic action between moulds and yeasts, the chief action of the former being apparently to secrete diastase and degrade the starch or starch products to fermentable sugars which can then be attacked by the yeast. The results of the experiments given above seem to indicate an action analogous to this supposed symbiosis.

[1] George Harris Morris, Ph.D., B.Sc., *Journ. Chem. Soc.*, vol. lxxix, p. 1085.

APPENDIX G

PREPARATION OF NUTRIENT SOLUTIONS, GELATINE WORT, &C.

Yeast Water is frequently used as a nutrient medium in conjunction with other substances, sugar, &c. 75 grams of pressed yeast are warmed in a water-bath, and to the softened yeast one litre of water is added and the whole brought to a temperature near boiling. It is then filtered through double filter papers, and should give a clear yellow solution. It may be subdivided into stock vessels and sterilized in them at a boiling temperature in a water-bath. 5 or 10 per cent. of sugar dissolved in this fluid gives a useful nutrient medium for yeast.

Plain Wort sterilized. To 1,000 c.c. of wort of specific gravity about 1,050–1,060 add 3 grams of dried egg albumen, heat gently till a very decided 'break' is produced, cool, filter (a small felt bag or jacketed copper funnel is desirable), sterilize in flasks or tubes, &c. by water-bath heat.

Wort Gelatine. A very good quality of thin sheet gelatine is necessary; from one to two ounces per pint of water is required according to the nature of the sheet gelatine. An experiment will decide what is best to use to obtain a certain consistency in the set gelatine. Break into small pieces and soak in cold water for a few hours, add the remainder of the volume of water hot, and digest the whole in a water-bath at about 160° F. till completely dissolved; now add a teaspoonful of raw egg-white well whipped with a little of the gelatine solution cooled, pour into the whole volume of gelatine and raise the temperature till the egg-white is quite coagulated and a decided 'break' has occurred. The temperature should be about 185° F. Filter hot through a felt bag or filter-paper in special jacketed funnel, collect in convenient vessels, flasks, test-tubes, &c., and re-sterilize at water-bath heat, say 180° F. To gelatine so prepared may be added a variety of nutrients; hopped wort or sweet wort may take the place of the water, or sugar may be added, or meat essences, e.g. Liebig brand, Valentine, &c.

Wood-Smith's Wort Gelatine for Yeast growing. Malt wort of specific gravity 1,100 is taken, and to it is added 1 per cent. of calcium chloride and 10 per cent. of the best gold-leaf gelatine, the whole being rendered alkaline to the extent of 0·5 per cent. of caustic soda. This mixture is heated until it is quite liquid, well shaken and filtered bright into vessels, flasks, Petri dishes, test-tubes, &c., in which it can be finally sterilized and left to set. The calcium chloride strengthens the yeast colonies, whilst the free alkali inhibits the growth of bacteria.

Solutions for nourishing the Bacteria which may be in Yeast Samples. Sometimes one wishes to deal with a specimen of yeast so as to discover what other organisms, e.g. bacteria, may be present. In such a case it is plain that, where the intrusive organisms are not perhaps 1 to 1,000 of the yeast cells, a liquid is needed in which the bacteria shall grow, and not the yeast. Such a liquid is not easily obtained; but such experiments as the author has made seem to indicate that gelatine prepared in the usual way, to which Brand's essence of meat or some similar preparation has been added to the extent of some 2 or 3 per cent. and carefully neutralized with calcium carbonate before the fining and filtering operations take place, answers very fairly. If the gelatine were made with a good meat broth it would probably serve as well, but this is much more troublesome. The great point in this case is to keep out sugar in any form, otherwise the yeast cells are certain to grow.

The student who wishes to acquaint himself with more of the practical details in connexion with the cultivation of yeast and bacteria is referred to the following:—

Journ. Chem. Soc. Ind., 1885, p. 698, Percy Frankland.
Ibid., 1886, p. 114, Percy Frankland.
Ibid., 1887, p. 113, G. Harris Morris.
Bacteriology, Crookshank.
Practical Bacteriology, Kanthack and Drysdale.

The last-named is a capital handbook for students' purposes.

APPENDIX H

FEHLING'S SOLUTION

Pure recrystallized Sulphate of Copper . .	34·64 grams
Rochelle Salt	173·0 „
Caustic Soda (NaHO)	60·0 „

Dissolve the Rochelle salt and the caustic soda together in about 400 c.c. of water, making up to 500 c.c. when cold. Powder the sulphate of copper in a mortar and dissolve in 400 c.c. of cold water, add 0·5 c.c. of concentrated sulphuric acid, and make up to 500 c.c. when solution is complete. Distilled water should be used. The solutions are to be kept in separate bottles and mixed in equal volumes when Fehling's solution is required, say 12 c.c. or 15 c.c. of each; mix well by shaking, or in any convenient way, then dilute and use according to the detailed method in Chap. VI.

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